International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Effect of Dentin Deproteinization using Bromelain Enzyme and Sodium Salt of Vitamin C on Microleakage in Class V Composite Restorations: An in Vitro Study

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Abstract: ***Aim: The aim of the present study was to evaluate the effect of surface treatments using sodium hypochlorite and sodium salt of Vitamin C when compared to Bromelain on microleakage of class V composite restorations. Methods and Material: 60 intact premolars were collected and class V cavity preparations were done on buccal surfaces and were divided into three groups of 20 each based on surface treatments done as Group A: acid etching alone, Group B: acid etching followed by hypo-sodium salt of Vitamin C, Group C: acid etching followed by Bromelain. Interfacial analyses of 5 randomly selected samples from each group were observed under Scanning electron microscope (SEM). Remaining 15 samples of each group were prepared for dye penetration and microleakage was analysed using stereomicroscope. Statistical analysis: statistical analysis was performed with PostHoc test and Kruskal Wallis using SPSS version 20. Results: The samples that were treated with Bromelain showed significantly least microleakage when compared to those treated with a combination of sodium hypochlorite- sodium salt of Vitamin C and acid etching alone. Conclusions: Bromelain surface treatment showed least microleakage with no gaps between the dentin and resin surface on observation under SEM.***

Keywords: *Bromelain, Microleakage, Scanning Electron Microscopy*

1. Introduction

Deproteinization of acid etched dentin using sodium hypochlorite (NaOCl) is a well accepted method of improving the permeability and wettability of dentin by eliminating the weakest collagen interface during bonding [1]. NaOCl in concentrations of 10%, 1.5%, and 5% for 2 minutes were recommended to produce collagen depleted apatite rich dentin [2]. Studies have shown significant improvement in bond strength of adhesive with least microleakage scores to deproteinised dentin [3]. Saboia et al stated that NaOCl treatment resulted in controversial results with some negative effects on the bond strength of dentin [4]. The decreased bond strength values were proposed due to the liberation of chloramines and reactive oxygen free radicals on lysis of pyridinoline crosslinks of type I and type II collagen which inhibits complete polymerization of dental adhesive [5].

Nagpal et al stated that 10% sodium salt of Vitamin C (prepared by adding 10gms of sodium ascorbate powder to 100ml of distilled water, pH - 7) nullifies the deleterious effect of NaOCl by restoring the redox potential [6]. Though deproteinisation with a combination of 5% NaOCl and 10%Ascorbate are acceptable, they still pose certain disadvantages like its cytotoxicity, caustic nature, unpleasant taste and odour.

One of the recently introduced deproteinising agents obtained from pineapple stem is Bromelain enzyme, commercially available as powder form (Bangalore sales, India) [7]. Bromelain is a mixture of different thiol endopeptidases and other components like phosphatase, glucosidase, peroxidase, cellulase, escharase, and several protease inhibitors [8]. Kirti Chauhan et al also stated that Bromelain enzyme resulted better bond strength when compared to NaOCl [9]. Dayem et al stated that Bromelain enzyme is 7 times more effective than NaOCl in their nanoleakage study but there has not been a comparative microleakage study evaluating the effect of combination of 5% hypo and 10%sodium ascorbate surface treatments with bromelain enzyme.

Hence, the aim of the present study is to compare the microleakage and study the resin dentin interface with the combined use of sodium hypochlorite-sodium ascorbate and more naturally available Bromelain.

2. Subjects and Methods

Sixty human premolars extracted for orthodontic treatment were collected for the study. The teeth were cleaned with ultrasonic scalers and stored in saline. Class V cavities were made on the buccal surfaces of premolars using an ISO 012 straight fissure diamond bur in an air-water cooled high speed hand piece. The cavity preparations were standardised with a width of 3mm mesiodistally, 2mm occlusogingivally and 2mm deep measured at the gingival level with the help of a periodontal probe, ensuring the axial wall to be always in dentin. The occlusal and gingival cavosurface margins

were prepared in enamel. The teeth were divided into two groups, Group I of 45 teeth for microleakage evaluation & Group 2 of 15 teeth for scanning electron microscopic evaluation of ultrastructure of resin tooth interface. Each group was subdivided into 3 subgroups (a, b, c) according to the surface treatments done.

Subgroup 1a & 2a: The teeth were etched with 37% phosphoric acid (Scotchbond Multi-purpose Etchant, 3M, USA) for 15sec and rinsed with water for 30 sec. The surface was blot dried with dry cotton pellet, leaving it visibly moist.

Subgroups 1b & 2b**:** 5.25% NaOCl (Nice Chemicals Pvt. Ltd, Coimbatore, India.) was applied for one minute to the acid conditioned cavity surface, followed by rinsing for 30 seconds to which 10% sodium ascorbate was applied for 1 minute. It is then rinsed for 30seconds. The surface was blot dried before bonding.

Subgroups 1c & 2c**:** The teeth were etched with 37% phosphoric acid for 15sec and rinsed with water for 30 sec. The surface was blot dried with dry cotton pellet, leaving it visibly moist. Bromelain enzyme (Bangalore sales corporation, India) was then applied using a disposable brush for a dwell time of 1 min and it was removed with 5 ml distilled water. The enzyme was washed off with distilled water.

All the samples were coated with fifth generation dental adhesive Prime & Bond NT (Dentspl, USA) and left undisturbed for 20 seconds then light cured for 10sec using light curing unit (Bluephase, Ivoclar Vivadent, Schaan, Lichtenstein) of intensity, 400 mW/cm2. The cavities were restored with nano composite Tetric N ceram (Ivoclar Vivadent Mumbai, India) using incremental technique and light cured for 30 seconds

Dye leakage test:
All the specimens of Group I were air dried and coated with two layers of nail varnish leaving 1mm of window around the cavity margins. The samples were then immersed in a freshly prepared 2% methylene blue dye for 5days. The teeth were then rinsed with water, the nail varnish removed and left to air dry at room temperature for 24hours. The teeth were sectioned longitudinally in a bucco-lingual direction by a cut through the centre of the restoration with the help of a diamond disk (Kerr Dental, USA). The degree of marginal leakage was determined by dye penetration, starting from the gingival margin of the restoration and moving towards the axial wall. Dye penetration at the tooth restoration interface was assessed by stereomicroscope (Olympus Corporation, USA) at 10X magnification.
According to Munro GA etal [4] the following scoring system was used to assess the degree of leakage and depth of dye penetration.
0 = no evidence of micro leakage
1 = dye penetration up to half of the cavity depth
2 = dye penetration of more than half the cavity depth
3 = dye penetration along the axial wall

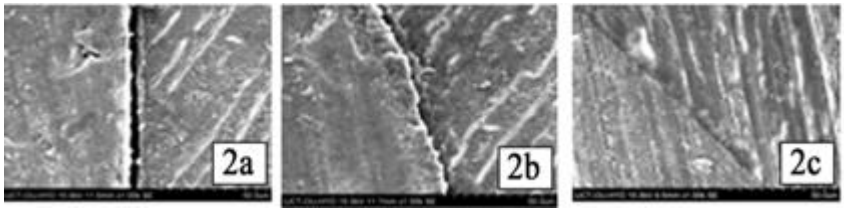
Specimen preparation for SEM:
All the specimens under Group II were sectioned in a bucco lingual plane through the centre of the restoration and polished. The specimens were mounted on alumina stubs and further dried in vacuum before gold sputtering. Gold sputtering was carried out under reduced pressure in an inert argon gas atmosphere in an argon sputter coater. The gold coated samples were examined under scanning electron microscope (JSM-6400V, JEOL, Tokyo, Japan) operated at 15Kv. Micrographs of the axial resin interface were taken at 1000X to observe the quality of bonding between the restoration and dental hard tissue.

3. Results

The results were statistically analysed by Kruskal Wallis and PostHoc test. When the three groups were compared with respect to microleakage the acid etched groups (Fig: 1a) and combination of sodium hypochlorite and sodium ascorbate treated groups (Fig: 1b) showed significantly higher leakage than Bromelain treated group (Fig: 1c). The results were statistically significant with $p<0.05$ with respect to micro-leakage scores by Kruskal Wallis by ranks (Table: 1). Subgroup 1a in comparison with (subgroup Ic) and (subgroup Ia) with (subgroup Ib) showed significant difference, $p<0.05$ with respect to PostHoc test (*$p<0.05$).

This study also observed the resin dentin interface after various surface treatments under scanning electron microscope this revealed that there is no gap between the resin and the dentin in the Bromelain treated group (Fig:2c) which supported the results obtained in evaluation of microleakage. For the acid etched teeth (Fig: 2a) which were not deproteinized there was no tubular penetration of the resin with the presence of generalized gap. For the sodium hypochlorite group (Fig: 2b) there was little tubular penetration with very few and short resin tags.

High resolution SEM micrograph of marginal adaptation at 1500x. Fig 2a: Acid Etching Alone. Fig 2b: Hypo And Sodium Ascorbate Treated Fig 2c: Bromelain Treated



Dye penetration of study samples Figure 1a: Acid Etching Alone. Figure 1b: Hypo And Sodium Ascorbate Treated. Figure 1c: Bromelain Treated

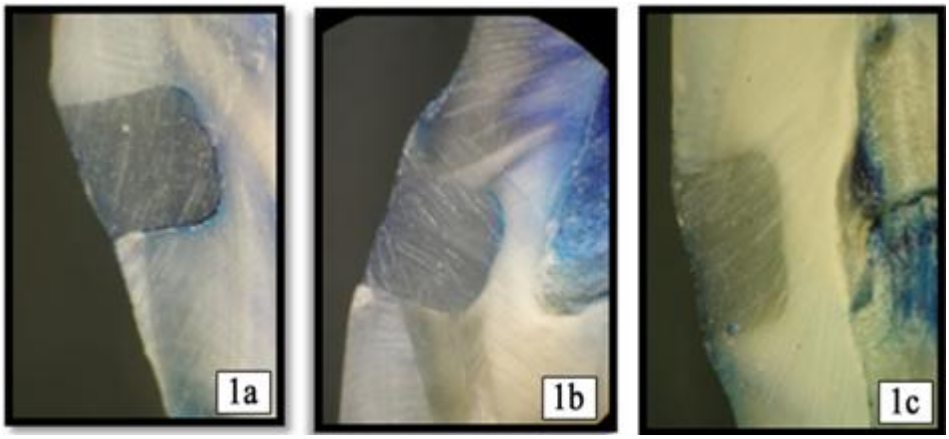
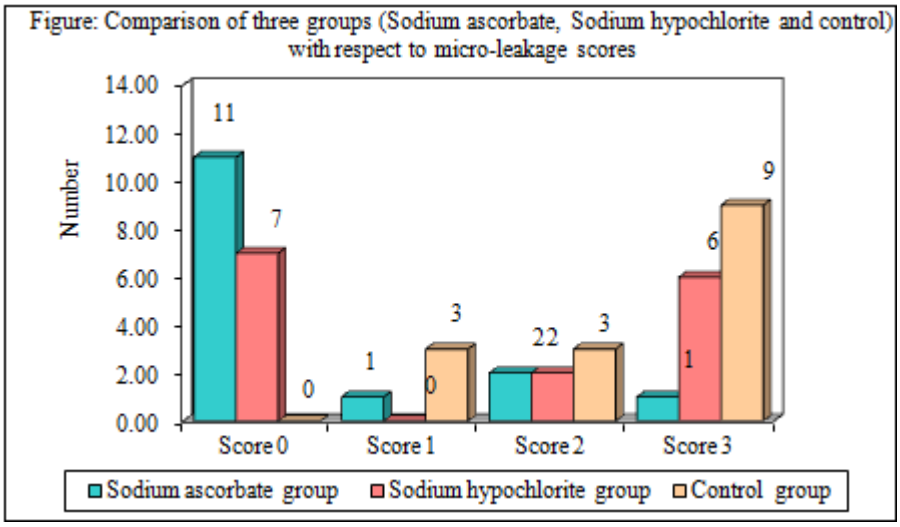


Table 1: Comparison of three groups (Sodium ascorbate, Sodium hypochlorite and control) with respect to micro-leakage scores by Kruskal Wallis ANOVA by ranks

Groups	Means	Std.Dev.	Median	Sum of ranks	H-value	P-value
Sodium ascorbate group	0.53	0.99	0	214.50	15.0126	0.0006*
Sodium hypochlorite group	1.47	1.46	2	343.50		
Control group	2.40	0.83	3	477.00		

*p<0.05



Graph 1

4. Discussion

Despite significant improvements of adhesive systems, the presence of collagen fibrils in dentin poses a clinical challenge in attaining successful adhesion [1, 10].

Deprotienization of the exposed collagen fibrils with proteolytic agents like NaOCl results in the formation of reverse hybrid layer [11]. This procedure increases the tubule diameter due to the loss of demineralised peitubular dentin. This substrate is rich in hydroxyapatite crystals as a result there is a more stable interface, essentially made of minerals. This is surface more or less acts like enamel with very insignificant amounts of protein. Studies have also suggested that collagen removal and application of adhesive resin directly on the exposed dentinal apatite significantly improves the bond strength and potentially overcomes the problem of hydrolytic degradation of non hybridized band of collagen [12].

In the present study, the concept of hybrid layer is completely eliminated with 3%NaOCl sol. NaOCl is a non-specific proteolytic agent which effectively removes the organic components from the biological materials at room temperature, the process known as deproteinization. Thus the surface is similar to that of etched enamel with high surface energy and increased wettability [1, 3, 5, 6]. Pimenta et al have demonstrated that NaOCl reduces the bond strength and increases the microleakage between resin composite and dentin [10]. Pashley et alattributed this fact to the presence of remnants and by-products of NaOCl exhibiting a negative effect on the polymerization of dental adhesive systems [11].

Nagpal et al used 10% sodium ascorbate In order to counteract the oxidizing affects of NaOCl. Sodium ascorbate, an anti oxidant or a reducing agent can cause the reversal of bond strengths of collagen depleted dentin [5]. Sodium ascorbate can interact with the by-products of NaOCl resulting in the neutralization and reversal of oxidizing effects of NaOCl-treated dentin surface. Sodium

ascorbate was used in the place of ascorbic acid to avoid the potential double etching effect of this mild acid on etched dentin [13]. It acts as a reducing agent and helps in restoring the redox potential of dentin from oxidized substrate to a reduced substrate thus facilitating complete polymerization. Pashley and others demonstrated that 5.25% NaOCl reduced the resin dentin bond strength but it can be reversed by 10%sodium ascorbate treatment for 1min [2].

Another deproteinizing agent used in the study was Bromelain, when applied on the conditioned dentin significantly decreased the microleakage scores. Raad et al demonstrated the ability of Bromelain enzyme to remove the collagen fibres from the acid etched dentin thus resulting in the increased permeability and high surface energy [7]. This resulted in spreading and diffusion of monomer through the dentin. At the same time use of Bromelain did not change other properties of dentin as sodium hypochlorite does. More over Bromelain is patient friendly when compared to sodium hypochlorite that has intolerable odour and taste [11].

As the concept of hybrid layer is completely eliminated in this study with the use of deprotenizing agents, the resulted surface is similar to that of etched enamel with increased wettability. In order to create this collagen depleted dentin, 5th generation total etch adhesive system, Prime & bond NT was used in the present study.

The marginal integrity was evaluated using two different parameters, that is, estimation of microleakage by dye penetration test using methylene blue under stereomicroscope and interfacial adaptation under SEM for better analysis.

5. Conclusion

Within the limitations of the study we conclude that there is reduced microleakage when the dentinal surface was treated with Bromelain when compared to the those of the specimens which were acid etched and NaOCl and sodium ascorbate surface treated.

Treating the dentin surface with more naturally, occurring Bromelain resulted in an intact restoration when compared to hypo and sodium ascorbate, which has negative effects on denitn.

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