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Effect of Various Surface Treatments on the Microleakage and Ultrastructure of Resin-tooth Interface

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Clinical Relevance

Under both moist and dry conditions, the application of sodium ascorbate on sodium hypochlorite-treated acid-etched dentin reduced microleakage and improved tubular penetration.

SUMMARY

This study evaluated the effect of collagen removal and sodium ascorbate treatment of acid-etched dentin on the microleakage and ultrastructure of resin-tooth interface under moist and dry conditions using an acetone-based 1 bottle adhesive system. Class V cavities were made on the buccal surfaces of 90 premolars scheduled for orthodontic extraction. The cavities were etched with 37% phosphoric acid (DPI tooth conditioning gel/India) for 15 seconds. The teeth were divided into six groups with 15 teeth each. In Group 1, the etched surface was blot dried with a dry cotton pellet, leaving it visibly moist, and Prime & Bond NT (Dentsply Detrey/Germany) was applied. In Group 2, after acid conditioning, the cavity surface was air dried for five seconds, followed by application of Prime & Bond NT. In Group 3, 3% NaOCl (Hyposol, Prevest Denpro Ltd/India) was applied to the acid-conditioned cavity surface for two minutes. The surface was blot dried before bonding. In Group 4, after NaOCl treatment, the surface was air dried for five seconds, followed by application of the bonding agent. In Group 5, 10% sodium ascorbate (chemically pure) was applied to the NaOCl-treated acid conditioned tooth surface for one minute. The surface was blot dried before bonding. In Group 6, after sodium ascorbate treatment as in Group 5, the cavity surface was air dried for five seconds before bonding. The cavities were restored with the hybrid composite Spectrum TPH (Dentsply Detrey, Konstanz, Germany). The teeth were extracted immediately after restoration, and the specimens were prepared for microleakage testing using 2% methylene blue dye and for scanning electron microscopic evaluation. The results of the dye penetration were analyzed with Kruskal-Wallis non-parametric analysis followed by the Mann-Whitney U test at a significance level of *p*=0.05. After acid etching, the conventional acid etched groups and groups with NaOCl treatment demonstrated extensive leakage. Sodium ascorbate treatment

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of the NaOCl-treated dentin significantly reduced microleakage. No statistically significant difference between moist and dry bonding was observed in all groups. Although resin tag penetration improved in both the NaOCl-treated and NaOCl/ascorbate-treated groups, an absence of gap at the resin dentin interface was observed only for the NaOCl/ascorbate-treated groups.

INTRODUCTION

The adhesive bonding of resin materials to etched enamel is a proven technique with long-term clinical success for the prevention of microleakage and the retention of esthetic restorative materials.[1] A pertinent factor in achieving satisfactory adhesion of resin composites to dentin is the way the dentin surface is treated before an adhesive is applied. Fusayama and others suggested removal of the smear layer from dentin using phosphoric acid treatment to improve adhesion.[2] Penetration of the adhesive resin into the exposed fibrillar collagen network of the demineralized dentin zone results in the formation of a micromechanical interlock known as the hybrid layer.[3] The collagen fibril network of demineralized dentin represents a soft, delicate bonding substrate that may contribute to the technique sensitivity of bonding procedures. For proper formation of the hybrid layer, water is essentially required to prevent collapse of the collagen fibers. However, over-wetting may lead to dilution or deterioration of the monomers, reduced final degree of cure and formation of water-containing defects within the adhesive layer.[4] If it is necessary to choose between over-drying or over-wetting of total-etched deep dentin, the former is to be preferred, since vital deep dentin is intrinsically wet after removal of the smear layer.[5] The optimum level of moisture required to maintain the integrity of collagen without compromising bonding cannot be clinically ascertained.

The stress absorbing role performed by the hybrid layer in dentin bonding has also been the subject of controversy, as considerably higher bond strengths after elimination of the collagen network have also been reported.[6-7] Spencer and Swafford have demonstrated that a demineralized, non-protected dentin zone remains beneath the hybridized layer due to incomplete resin infiltration.[8] Subsequent hydrolysis of the exposed collagen peptides could lead to degradation of the dentin to resin bond, resulting in decreased bond strength and increased microleakage over time.[9]

Because of the limitations associated with the use of total-etch systems, research has been directed toward techniques that alter the chemical composition of dentin by removing the collagen from dentin to enhance resin bonding. The removal of unsupported collagen fibers could have a beneficial effect on the primer, adhesive spreading and diffusion through dentin.[10] It may produce a more durable adhesion to the hydroxyapatite component of the dentin substrate.

NaOCl is a non-specific proteolytic agent that effectively removes organic compounds at room temperature and has been frequently applied for collagen removal with controversial results.[11-12] Improvement in adhesion to demineralized and deproteinized dentin has been reported, but no interference and decrease in adhesion have also been reported.[6-7,13-15] This decrease in bond strength after NaOCl application has been attributed to the loss of the shock absorbing effect of the hybrid layer or the reduction in the physical properties of dentin.

However, as the application time of NaOCl is minimal, some recent studies have related this interference mainly to the effect of the remaining NaOCl on the polymerization of resin and the specificity of different adhesive systems to the oxidizing effect of sodium hypochlorite.[16-18] This residual NaOCl could be neutralized by the application of sodium ascorbate to the oxidized dentin, which acts as a reducing agent, restoring the redox potential of dentin and converting the microenvironment of the dentin from an oxidized substrate to a reduced substrate, thus facilitating complete polymerization. Lai and others demonstrated that a reduction in bond strength caused by NaOCl could be reversed by the application of 10% sodium ascorbate, a neutral biocompatible antioxidant, for one minute.[16] However, sodium ascorbate, when applied directly on dentin without prior sodium hypochlorite treatment, has been found to reduce bond strength. End products formed by the reaction between sodium ascorbate and sodium hypochlorite are oxalic acid and L-threonic acid, both which are water soluble.[19] It is possible that, by restoring the altered redox potential of the oxidized bonding substrate, sodium ascorbate allows free radical polymerization of the adhesive to proceed without premature termination and, hence, reverses the compromised bonding in NaOCl or H_2O_2 treated dentin.[16]

Although many *in vitro* studies have evaluated the effect of surface pre-treatments and compared the efficacy of moist and dry bonding techniques, the combined effect of moisture, collagen removal and reducing agent application on dentin bonding *in vivo* is still unclear. This study investigated the effect of collagen removal and sodium ascorbate treatment of phosphoric acid etched dentin on the microleakage and ultrastructure of resin tooth interface under moist and dry bonding conditions using an acetone-based 1 bottle adhesive system. The null hypothesis tested was that there is no significant effect of moisture level, NaOCl and sodium ascorbate treatment on resin-dentin bonding.

METHODS AND MATERIALS

This study was performed in 90 caries free premolars, scheduled for orthodontic extraction, after receiving the patient's informed consent. Class V cavities were made on the buccal surfaces of the premolars using an ISO 012 straight fissure diamond bur (Dentsply Detrey) in an air-water cooled high speed handpiece. No local anesthesia was used. The cavity preparations were standardized with a width of 3 mm mesiodistally, 2 mm occluso gingivally and 1.5 mm deep, measured at the gingival level, ensuring the axial wall to always be in dentin. The occlusal and gingival cavosurface margins were prepared butt joint in enamel without any bevel. No additional mechanical retention was placed. The teeth were divided into six groups of 15 teeth each according to different surface treatments and the degree of moisture of the bonding substrate.

Group 1 (Moist, M): The cavities were etched with 37% phosphoric acid for 15 seconds and rinsed with water for 30 seconds. The surface was blot dried with a dry cotton pellet, leaving it visibly moist. One coat of bonding agent Prime & Bond NT (Dentsply Detrey) was applied with a bristle brush and left undisturbed for 20 seconds, air-thinned for five seconds, then light cured for 10 seconds at a light intensity of 600 mW/cm^2.

Group 2 (Dry, D): After acid conditioning, the cavity surface was air dried for five seconds with compressed air from an air syringe, the tip of which was kept at a distance of 2 cm from the prepared tooth. Subsequently, Prime & Bond NT was applied, as for Group 1.

Group 3 (Moist Hypochlorite, MH): Three percent NaOCl was applied for two minutes to the acid conditioned cavity surface, followed by rinsing for 30 seconds. The surface was blot dried before bonding.

Group 4 (Dry Hypochlorite, DH): After NaOCl treatment, the surface was air dried for five seconds, followed by an application of the bonding agent.

Group 5 (Moist Hypochlorite Ascorbate, MHA): Ten percent sodium ascorbate was applied for one minute to the NaOCl-treated, acid conditioned tooth surface, then rinsed for 30 seconds. The surface was blot dried before bonding.

Group 6 (Dry Hypochlorite Ascorbate, DHA): After sodium ascorbate treatment as in Group 5, the cavity surface was air dried for five seconds before bonding.

The cavities were bulk filled with hybrid composite Spectrum TPH (Dentsply Detrey) and light cured for 40 seconds at 600 mW/cm^2. The restorations were polished using Enhance system disks (Dentsply Detrey). The teeth were extracted immediately after restoration, cleaned and stored in distilled water for between two to four weeks. Ten samples from each group were used for evaluation of microleakage and five for scanning electron microscopic examination.

Dye Leakage Test

In preparation for the dye penetration test, the specimens were air dried and coated with two layers of sticky wax, leaving a 1 mm window around the cavity margins. The samples were then immersed in an inverted fashion in a freshly prepared 2% methylene blue dye for five days. The teeth were then rinsed with water, the sticky wax removed and the teeth left to air dry at room temperature for 24 hours. The teeth were sectioned longitudinally in a buccolingual direction by a cut through the center of the restoration. 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 at 10x magnification by two independent precalibrated examiners who were unaware of the treatment groups. In case of any disagreement, new readings were performed until a consensus was reached. The following scoring system was used:[20]

Degree of Leakage Depth of Dye Penetration

0 = no evidence of microleakage

1 = dye penetration up to half the cavity depth

2 = dye penetration of more than half the cavity depth

3 = dye penetration along the axial wall

Specimen Preparation for Scanning Electron Microscopy

For scanning electron microscopic evaluation, the specimens were sectioned vertically in a buccolingual plane through the center of the restoration and polished with Enhance system disks and pastes containing 1 µm and 0.03 µm alumina particles (Dentsply Detrey), similar to the study by Wang and Spencer, who used 1 µm and .05 µm alumina particles.[21] The sections were fixed in 10% formalin for 24 hours and decalcified in 6N HCl for 30 seconds, rinsed in distilled water and deproteinized by 10-minute immersion in 1% NaOCl, then rinsed in distilled water. After acid base treatment, the specimens were subjected to dehydration in ascending grades of ethanol up to 100% (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 95% for 30 minutes and 100% for 60 minutes). The specimens were mounted on aluminum stubs and further dried in vacuum before sputter coating with gold. Gold Sputter Coating was carried out under reduced pressure in an inert argon gas atmosphere in an Agar Sputter Coater P7340 (Agar Scientific Ltd, Essex, England). The gold-coated samples were examined under scanning electron microscope (Leo 435 VP, Cambridge, UK) operated at 15kV. Micrographs of the axial resin dentin interface were taken at 1000x to observe the quality of bonding between the restorations and dental hard tissue.

Statistical Analysis

The results of dye penetration were analyzed with Kruskal-Wallis non-parametric analysis followed by Mann-Whitney U test to evaluate differences among the experimental groups at a significance level of p=0.05.

RESULTS

Dye Leakage Study

Microleakage scores are presented in Table 1. Both the conventional acid-etched groups and the acid-etched NaOCl-treated groups demonstrated extensive leakage in both moist and dry conditions (p>.05). Sodium ascorbate treatment of the deproteinized dentin significantly reduced the microleakage as compared to the conventional acid-etched groups and acid-etched NaOCl-treated groups, with no statistically significant difference between moist and dry bonding.

Scanning Electron Microscopy

SEM findings are summarized in Figures 1-6. For specimens that had been acid-etched but not deproteinized, there was little or no tubular penetration, with very few and short resin tags with the presence of generalized gap. For etched and NaOCl-treated samples, deeper tubular penetration and filled lateral tubular branches were observed, but some areas still showed an interfacial gap. Prime and Bond NT showed better interfacial adaptation, deeper tubular penetration and filled lateral tubular branches when used after NaOCl/sodium ascorbate treatment.

DISCUSSION

In tooth colored restorative materials, early loss of restoration is no longer a clinical problem. However, marginal leakage and consequental marginal discoloration remains the most frequent reason to replace/repair an adhesive restoration.[22] Additionally, a "retained" restoration is always assessed as "bonded" to the cavity walls, yet, retention and bonding are two different concepts. A composite restoration might be retained in a Class V cavity without being totally bonded at the resin dentin interface.[23] Therefore, besides bond strength, testing of marginal sealing effectiveness of adhesive is needed.

In this study, the air drying protocol for Prime & Bond NT was used for experimental comparison, dissimilar *in vitro* conditions, as there is continuous transudation of dentinal fluid due to intrapulpal pressure in vital dentin that has a magnitude of 25 to 30 mm Hg or 34 to 40 cm of water.[24-26] The presence of this intrinsic source of moisture may be all that is required to maintain the demineralized collagen matrix in its hydrated state.[27] Moreover, as water is required to maintain the collagen in its expanded state, in groups with sodium hypochlorite treatment, removal of the collagen fibers with deproteinizing agents would facilitate the access of resin to a substrate that is more permeable and less sensitive to moisture.[28]

Table 1: *Leakage Scores Observed in Different Treatment Groups*

Restorative Groups	Dye Leakage Scores 0	1	2	3
M^{α}	0	0	0	10
D^{α}	0	0	0	10
MH^{α}	0	2	2	6
DH^{α}	0	2	0	8
MHA^{β}	6	2	2	0
DHA^{β}	2	6	2	0

M-moist D-dry H-sodium hypochlorite A-sodium ascorbate.
Same superscript letters indicate no statistically significant difference at a significance level of p=*0.05.*

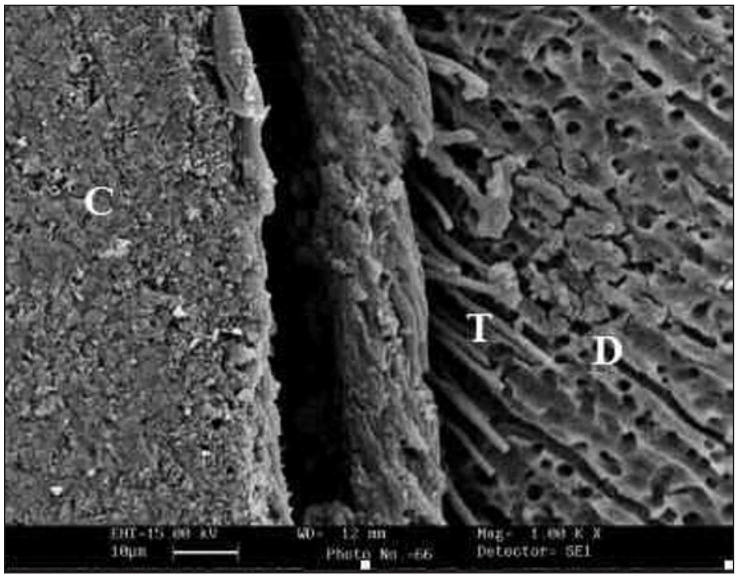

Figure 1. *Cross-section of the interface obtained* in vivo *between Prime&Bond NT and acid-etched, blot-dried dentin. Generalized gap can be observed with very few and short resin tags [Gp 1 (M)]. D= dentin, C=composite, T= resin tags.*

The results of this *in vivo* study demonstrated extensive leakage and generalized gap formation in samples restored with the hybrid composite Spectrum TPH and Prime & Bond NT without any surface treatment in both moist and dry conditions. These results are contrary to the previous *in vitro* study reporting a significant gap-free attachment of Prime & Bond NT to dentin following the manufacturer's instructions.[29] Most of the previous studies, which have reported high bond strengths, have been performed *in vitro* on flat dentin surfaces.[30-31] The current study has been performed in *in vivo* conditions in high C-factor cavities that could possibly explain the different results. Moreover, the environmental conditions might have also affected the results. It has been suggested that the hydrostatic pulpal pressure, the dentinal fluid flow and the increased dentinal wetness in vital dentin can affect the intimate interaction of certain dentin adhesives with dentinal tissue.[32-34] These findings are supported by studies that demonstrated low bond strength

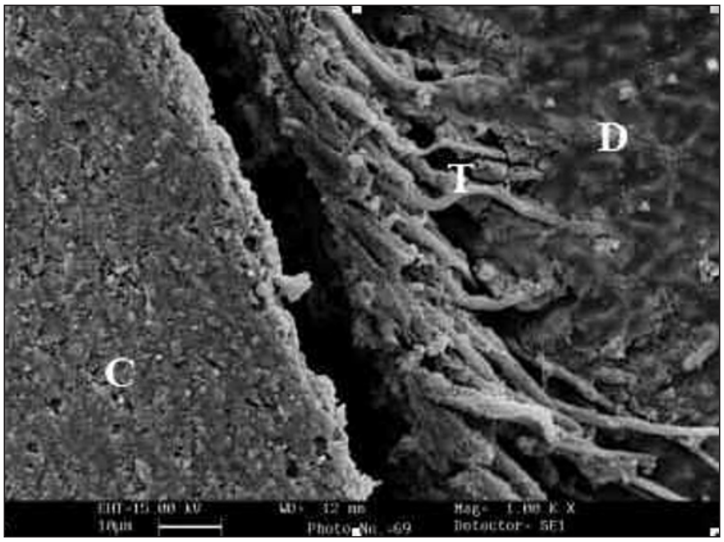

Figure 2. *Cross-section of the interface obtained* in vivo *between Prime&Bond NT and acid-etched, air-dried dentin depicts the presence of gap with little or no tubular penetration [Gp 2 (D)]. D= dentin, C=composite, T= resin tags.*

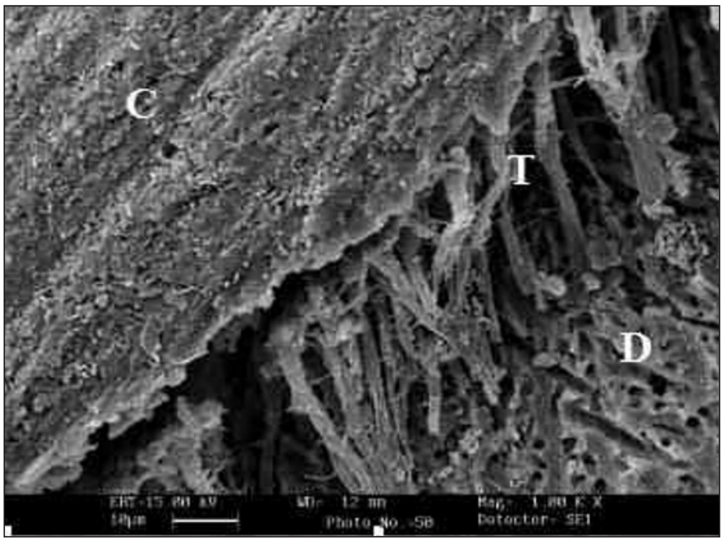

Figure 3. *Cross-section of the interface obtained* in vivo *between Prime & Bond NT and acid-etched, NaOCl treated, blot-dried dentin. Although resin tags and filled lateral tubular branches are numerous, some areas still show an interfacial gap. [Gp 3 (MH)]. D= dentin, C=composite, T= resin tags.*

in simulated clinical conditions maintaining intrapulpal pressure.[35] Hannig and Friedrichs also observed that a completely gap-free adaptation between composite and dentin could not be achieved reproducibly and consistently, either *in vivo* or *in vitro*, in cavities with a high C-factor.[36] They found that Prime & Bond 2.1 gave better results when applied *in vitro*, as compared to *in vivo* restorations. Median percentages for the *in vivo*/*in vitro* frequency of interfacial gap formation were 40% and 5.3%, respectively.[36]

No statistically significant difference in microleakage between blot-drying and air-drying, as observed in this study, is contrary to studies that reported moist bonding was superior to dry bonding *in vitro* and *in vivo*.[37-38] Other *in vitro* studies have also reported high bond strength to moist dentin than to air-dried acid etched dentin.[39-40] The different results in these studies could be related to the difference in the degree of moisture of dentin due to either *in vitro* conditions or to the compromised blood supply of periodontally compromised teeth *in vivo*.

The results of this study, however, are consistent with studies reporting microleakage in all the groups at both the enamel and dentin margins in Class V cavities with no statistically significant difference between moist and dry bonding.[41-42] Cardoso and others also reported similar bond strength to moist and dry dentin using an acetone-based adhesive system.[43]

In accordance with previous studies, specimens with NaOCl treatment showed deeper resin tag penetration into dentinal tubules as compared to specimens with no NaOCl treatment.[6,44-45] Extensive leakage was still observed after NaOCl treatment for both moist and dry bonding groups. The results may be explained by assuming that whatever improvement in marginal sealing effectiveness was expected because deproteinization was probably negated because of partial inhibition of polymerization at the resin dentin interface due to the oxidizing effect of the residual NaOCl or altered chemical structure of dentin after NaOCl treatment. Pyridinoline crosslinks, which occur in collagen Type I and Type II, were found to be disrupted by NaOCl with the formation of chloramines and protein-derived radical intermediates.[46-47] The presence of these reactive, residual, free-radicals in NaOCl-treated dentin may compete with the propagating vinyl-free radicals generated during light activation of the adhesive, resulting in premature chain termination and incomplete polymerization. It has been speculated that areas with unpolymerized resin may cause leakage.[48] This, in conjunction with other factors, such as high C-factor and moisture level of *in vivo* conditions, might have resulted in extensive leakage. Perdigão and others reported that NaOCl treatment decreased bond strength in spite of deeper penetration of resin.[14]

Results of this dye leakage study support studies that reported no difference in microleakage, decreased marginal adaptation or increased leakage after NaOCl treatment.[15,49-50] However, the results of this study are contrary to studies that reported a decrease in microleakage and improved marginal adaptation after NaOCl treatment in Prime & Bond 2.1.[12,51] This may be because demineralization and deproteinization of dentin could be influenced by various factors present in different studies, such as adhesive system composition, depth (superficial vs deep dentin), type of dental substrate (vital vs non-vital dentin), cavity configuration, sensitivity of the adhesive systems to the oxidizing effect of NaOCl and variation in the concentration and duration of application of NaOCl. Although Tanaka and Nakai have recommended 10% NaOCl as ideal concentration for dentin treatment, Prati and others reported complete removal of collagen fibrils, with concentration as low as 1.5% NaOCl for two minutes, while Toledano and others used 5% NaOCl for two minutes.[13,49,52]

Sato and others evaluated the effect of NaOCl treatment of etched air-dried dentin *in vitro* and reported that decreased bond strength of air-dried dentin increased after NaOCl treatment but observed the highest bond strength with wet bonding.[53] The current study revealed no significant difference in dye leakage between moist and dry bonding after deproteinization with leakage in both groups. This could be attributed to the effect of NaOCl on the polymerization of resin, which seems to be a more crucial factor influencing bonding rather than the moisture level of the substrate.

The results of this study depicted a significant reduction in microleakage after sodium ascorbate treatment of the collagen depleted dentin. These findings could be related to reversal of the adverse effects of NaOCl on the polymerization of resin composite by sodium ascorbate.

Since Vitamin C (ascorbic acid) and its salts are nontoxic and widely used in the food industry as antioxidants, it is unlikely that their use on dentin will create any adverse biological effect or clinical hazard.[16] Sodium ascorbate (pH 7) was used in place of ascorbic acid (pH 4) in this study to avoid the potential double etching effect of this mild acid on etched dentin.

This study observed no significant difference in microleakage with moist or dry bonding in deproteinized ascorbate-treated groups. As there would be no collagen network present on acid-conditioned NaOCl-treated dentin to interfere with resin infiltration, even air drying would not have adversely affected the resin dentin bonding. Acid-etching changes dentin to a hydrophobic surface with reduced surface energy.[54] Collagen removal by NaOCl treatment increases the wettability of the dentin surface, because deproteinization results in a hydrophilic surface and chemical interactions between the resin and deproteinized dentin are more likely to occur.[55] Elimination of the exposed collagen using NaOCl allows for the achievement of a rougher, porous surface similar to enamel, which allows for good mechanical retention and is less sensitive to water content, hence, reducing technique sensitivity.[55]

Within the limits of this *in vivo* study, it can be postulated that stability of the internal seal between resin composite and acid-conditioned dentin in cavities with a high C-factor is still questionable, and the optimum dentin surface pre-treatment still needs to be determined. The current study did not include caries affected dentin, effect of different concentrations of sodium hypochlorite and the long-term clinical evaluation of the restoration. In order to improve the longevity of adhesive resin composite restorations, further efforts should be directed to an improvement in the adhesive and bonding properties of filling materials placed on the dentin.

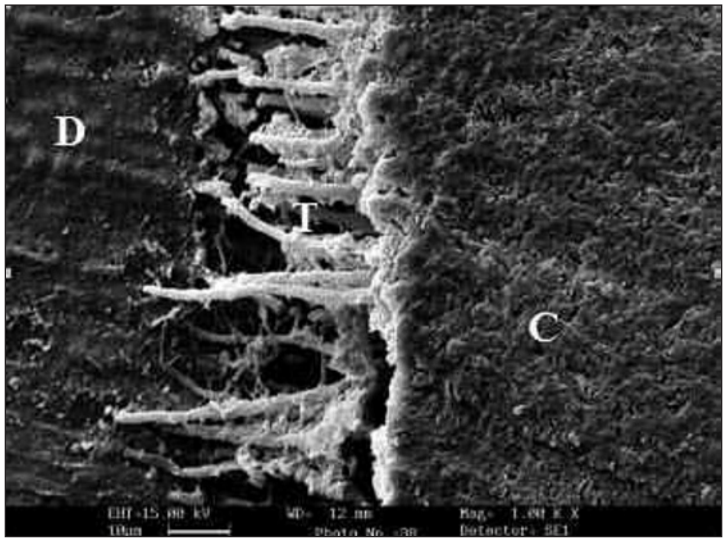

Figure 4. *Cross-section of the interface obtained* in vivo *between Prime & Bond NT and acid-etched, NaOCl treated air-dried dentin. Detachment above the resin tags can be observed in some areas. [Gp 4 (DH)]. D= dentin, C=composite, T= resin tags.*

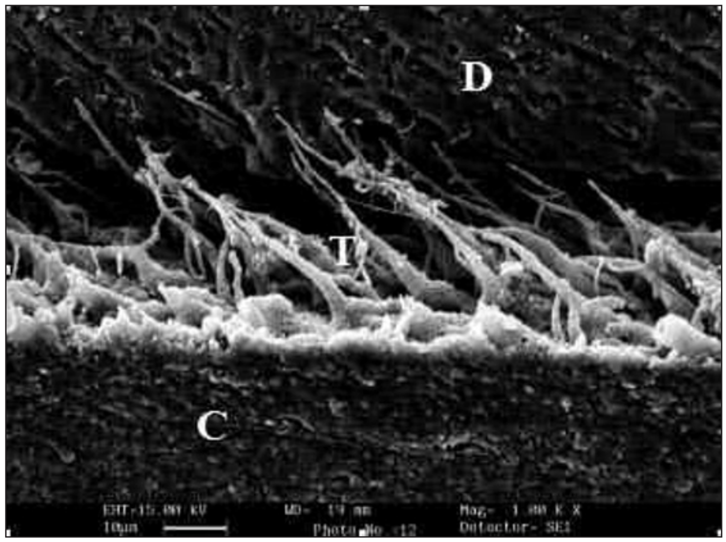

Figure 5. *Cross-section of the interface obtained* in vivo *between Prime&Bond NT and acid-etched, NaOCl treated, sodium-ascorbate treated, blot-dried dentin revealing penetration of resin tags into the tubules and into tubular anastomoses. [Gp 5 (MHA)]. D= dentin, C=composite, T= resin tags.*

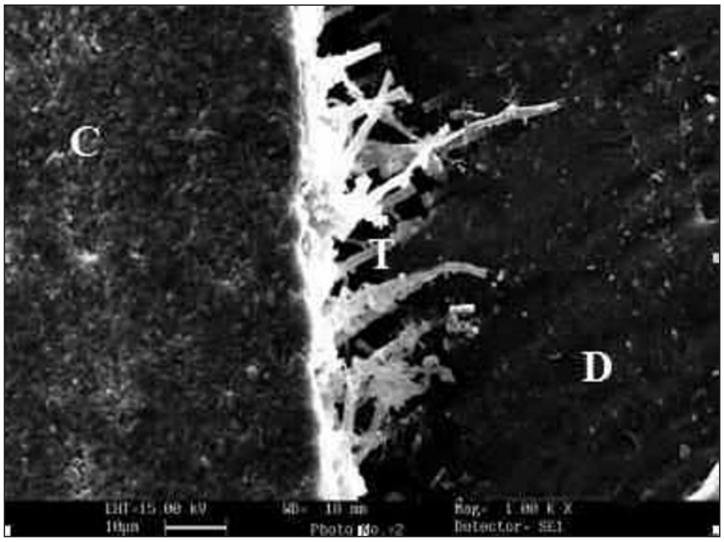

Figure 6. *Cross-section of the interface obtained* in vivo *between Prime&Bond NT and acid-etched, NaOCl treated, sodium-ascorbate treated, air-dried dentin shows improved tubular penetration and interfacial adaptation [Gp 6 (DHA)]. D= dentin, C=composite, T= resin tags.*

CONCLUSIONS

Restoration with Spectrum TPH and Prime & Bond NT does not prevent microleakage in Class V cavities *in vivo*. Sodium hypochlorite treatment, followed by sodium ascorbate treatment, significantly reduced microleakage. There was no statistically significant difference between blot drying and air drying in all the groups in this *in vivo* study. Sodium ascorbate/sodium hypochlorite treatment resulted in deeper tubular penetration and better interfacial adaptation at the resin dentin interface.

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