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# Effect of Deproteinising Agents on The Adhesion of Composite Resin To Dentin in Total Etch and Self Etch Techniques - A Nanoleakage Study

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## Abstract

**Background:** Despite advances in adhesive dentistry, the integrity of the hybrid layer at the dentin–restorative interface is of paramount importance for the long term bond durability of the restoration. Deproteinizing agents such as bromelain and ficin may enhance bonding by removing exposed collagen fibrils, however their effect on nanoleakage with different adhesive systems is not well established.

**Aim:** The effect of deproteinising agents on the adhesion of composite resin to dentin in total etch and self-etch techniques by evaluating nanoleakage.

**Materials and Methods:** Sixty extracted human molars were taken and standardized Class V cavities on the buccal surface were prepared. Samples were divided into three groups: Group I - control (no deproteinizing agent), Group II - 10% bromelain, Group III - 2% ficin. Each group was further subdivided into Sub-group A - self-etch and Sub-group B - total-etch adhesive subgroups. Based on the group deproteninzing agents were used and their respective bonding agents were applied. Restorations were completed with nano-hybrid composite resin. Nanoleakage was assessed by assessing rhodamine

B dye penetration using confocal laser scanning microscopy.

**Results:** Bromelain-treated groups demonstrated the least nanoleakage, with a significant reduction under the total-etch technique. Control groups showed moderate leakage, while ficin consistently exhibited the highest nanoleakage values with minimal improvement across etching modes.

**Conclusion:** Application of deproteinizing agents before bonding significantly reduced nanoleakage. Bromelain showed better results compared to ficin and controls, indicating its potential as an effective deproteinizing agent to enhance dentin bonding durability

**Keywords**: Deproteinizing agent, Bromelain, Ficin, Dentin bonding, Nanoleakage

## Introduction

Adhesive Dentistry is evolving everyday with Dentin Bonding Systems being continuously developed, bringing tremendous changes in the field of restorative dentistry. But despite the efforts, the bonded interface between dentin surface and the restorative material remains as the susceptible and weakest area.[1] During any restorative procedure, dentin etching is a complex procedure as it leaves the collagen fibers exposed along with the formation of smear layer.[2] Underneath this demineralized dentin zone, a partially demineralized zone and followed by nonetched dentin is seen.[3] After dentin etching the collagen fibrils are exposed. On polymerization, low-viscosity monomers fill the nano-cavities of this demineralized dentin, thereby forming a resin-reinforced and acid resistant layer called Hybrid layer or Interdiffusion zone.[4] Successful dentin bonding depends upon the hybrid layer formation which is sufficiently infiltrated with adhesive resins.

The Total Etch System helps in simultaneous removal of the smear layer from both enamel and dentin surfaces, followed by application of a single solution of primer and adhesive.[5] Thus, showing stable bonds between the composite resin and restorative surfaces.

Self-etch adhesive systems contain acidic co-monomers that help to simultaneously demineralize and infiltrate dentin, helps keep the smear plugs intact.[6] The present day clinicians prefer the all-in-one single bottle self-etching adhesive systems, as they are said to be the most promising adhesive approach for a durable restoration. They are highly hydrophilic polymers, permeable to water movement.[7]

A deproteinizing agent, capable of removing organic substances like the exposed collagen fibrils after dentin etching, is recommended.[10] The removal of this collagen mesh helps in better dentin-adhesive system interaction because of increased surface wettability. This protocol exposes the superficial dentin layer which has similar characteristics of etched enamel surface like greater presence of hydroxyapatite crystals with high surface energy, improving adhesive strength and therefore the adhesion.[11,12]

Incomplete penetration of resin within the hybrid layer leads to nanoleakage. The term ‘nanoleakage’ was coined by Sano et al in 1995.[8] In this phenomenon, leakage is seen as submicron porosities in lateral channels at the base of hybrid layer which have not been infiltrated with adhesive resin or have been poorly polymerized.[9] Hence, this study aims to evaluates the influence of deproteinising agents on the nanoleakage phenomenon, which would be studied using two different bonding systems.

**Aim & Objective**

The effect of deproteinising agents on the adhesion of composite resin to dentin in total etch and self-etch techniques by evaluating nanoleakage

**Materials and Methods**

**Preparation of the Samples:**

Extracted human permanent molars (n=60) were used in this study. Samples free of caries, restorations, cracks or obvious defects were included in this study. The samples were stored in 50% ethanol at 83C for a one month after extraction to avoid any kind of microbial contamination of the samples. This storage medium was chosen because it has only minor consequences on the dentin permeability [13].

Standardized class V cavities were prepared in all the samples (n=60) in the dimensions of 3x3 mm, 2 mm deep, on the buccal surface using cylindrical diamond burs (2837 UC 014 8 FG Cylinder, Midwest Diamonds, Dentsply Professional) in a high-speed handpiece and hand cutting instruments (hoe-#20 SE Hoe; 14-6-8, Hu-Friedy) was used to provide adequate finishing of the cavity. The cervical restoration margins were kept in dentin or enamel.

The teeth samples were randomly divided into three major groups (n=20) based on the deproteinising agent to be applied and further into two sub-groups (n=10) according to the dentin surface treatment. Table 1 depicts the composition of the adhesive system used in the study.

- Group I - Control group (without deproteinising agent)
- Group II - Application of 10% Bromlain
- Group III - Application of 2% Ficin
- Sub-group A - Self-etch bonding system
- Sub-group B - Total-etch bonding system

Table 1: Composition of the Prime & Bond Universal adhesive system

| Resin monomers | Initiator system | Solvent |
|---|---|---|
| PENTA (dipentaerythritol pentacrylate phosphate), 10-MDP (10-methacryloyl oxydecy] dihydrogen phosphate), Active Guard Technology crosslinker | CO/tertiary amine | 10-24.5% Isopropanol, 5-24.5% water |

**Group I (Sub-group A) - without Deproteinising Agent, Using Self-etch System**

All the samples of Group I (n=10) were uniformly bonded with the adhesive. Adhesive was then agitated for 20 seconds. The solvent was evaporated thoroughly by air blowing until there was no movement of the adhesive (at least for 5 seconds) according to manufacturer's recommendations. The surfaces were then cured for 20 seconds using light curing unit.

**Group I (Sub-group B) - without Deproteinising Agent, Using Total-etch System**

All the prepared dentinal surfaces of teeth (n=10) were etched with 36% phosphoric acid (Dentsply, DeTrey GmbH 78467 Konstanz, Germany) for 15 seconds and rinsed. Excess water was removed, but surface was not desiccated. The resultant surfaces were wetted. Bonding agent was left undisturbed for 20 seconds according to manufacturer's recommendations after which solvent was evaporated with air. Surfaces were cured using light curing unit (QHL 75 curing light, Dentsply Caulk, Milford, CT, USA) for 20 seconds.

**Group II (Sub-group A) - Application of 10% Bromelain, Using Self-etch System**

All the prepared dentinal surfaces of teeth (n=10) were treated with 10% bromelain for a minute, then water rinsed for 30 seconds, and blotted dry with tissue paper. The surfaces of teeth were uniformly bonded with the adhesive in the same way as Group I (Sub-group A).

**Group II (Sub-group B) - Application of 10% bromelain, Using Total-etch System**

The prepared dentinal surfaces of teeth (n=10) were etched and bonded in the same way as Group I (Sub-group B), but prior to the etching of the teeth in this group, 10% Bromelain was applied for a minute then water rinsed for 30 seconds, and blotted dry with tissue paper.

**Group III (Sub-group A) - Application of 2% Ficin, Using Self-etch System**

All the prepared dentinal surfaces of teeth (n=10) were treated with 2% Ficin for a minute, then water rinsed for 30 seconds, and blotted dry with tissue paper. The surfaces of teeth were uniformly bonded with the adhesive in the same way as Group I (Sub-group A).

**Group III (Sub-group B) - Application of 2% Ficin, Using Total-etch System**

The prepared dentinal surfaces of teeth (n=10) were etched and bonded in the same way as Group I (Sub-group B), but prior to the etching in this group, 2% Ficin was applied for a minute then water rinsed for 30 seconds, and blotted dry with tissue paper.

The cavities were then restored with (Ceram X Nano ceramic restorative) composite resin in incremental technique, light cured with light intensity of 500mW/cm$^2$ for 20 seconds, as directed by the manufacturer. Then samples were tested for nanoleakage using confocal laser scanning microscopy (CLSM) technique.

For the dye penetration test, all teeth were stored in a 50% alcoholic solution of 1% rhodamin-B-isothiocyanate (#12482; Merck, Darmstadt, Germany) for 24 h at 20$^{O}$C. After rinsing with water for 10 s, the teeth were embedded in methacrylate and sectioned parallel to the long axis of the tooth using a microtome saw (Leica 1600; Leica, Bensheim, Germany), separating the restorations into two parts. Prior to CLSM inspection, all specimens were stored in water for 24 h at 20$^{O}$C and kept humid throughout the whole experiment.

The measurements were carried out with a Leica Diaplan CLSM equipped with oil immersion objectives (100T, NA~1.3, resolution8250 nm; 40T, NA~1.0, resolution8500 nm; 10T, NA~0.45) and an Ar/Kr-mixed gas laser source (adjustable up to 40 mW). The laser was operated with a 510 nm short-pass filter (excitation maximum at 488 nm). In fuorescent mode, the reflected and scattered light was filtered with a 590 nm long-pass filter. Histotomographic images were recorded on wet specimens 10 mm deep parallel to the section surface. In the case of a microleakage (gap between dentin and composite visible under CLSM using 40x objective), teeth were excluded from the study. For lateral calibration, a silicon testing grid (Plano, Marburg, Germany) with a certified line width of 8.87¡0.01 mm was used. The lengths of dye penetration from the outer tooth surface towards the bottom of the cavity, representing the amount of nanoleakage, were measured at low magnification (10x objective). Therefore, we used an over-modulated signal of the fuorescent light to facilitate the measurement. Standard settings for contrast, brightness, size of confocal pin- hole and laser power were used for all the experiments. The data was recorded.

**Result**

The comparative analysis of nanoleakage values across the groups demonstrated substantial variability influenced by the treatments and etching modes applied. The Control group (Group I) consistently exhibited higher levels of nanoleakage compared to the 10% Bromelain group (Group consistently showed the lowest nanoleakage values among all groups (Table 2) (Fig 1).

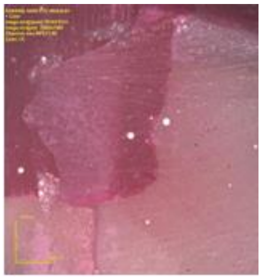

Figure 1: 2% Ficin group

Table 2: Comparison of mean Nano-leakage (in nm) in Total Etch mode b/w 3 groups

| Groups | Etch | N | Mean | SD | Mean Diff | p-value |
|---|---|---|---|---|---|---|
| Control | Self-Etch | 10 | 1423.18 | 95.48 | 24.83 | 0.65 |
| | Total Etch | 10 | 1398.35 | 138.08 | | |
| 10% Bromelain | Self-Etch | 10 | 1064.99 | 55.99 | 682.18 | <0.001* |
| | Total Etch | 10 | 382.81 | 68.05 | | |
| 2% Ficin | Self-Etch | 10 | 2265.11 | 124.21 | 142.25 | 0.01* |
| | Total Etch | 10 | 2122.86 | 108.82 | | |

Meanwhile, the 2% Ficin group (Group III) displayed the highest nanoleakage levels relative to both the Control (Group I) and 10% Bromelain groups (Group II), underlining its limited efficacy in reducing leakage (Table 2) (Fig 2 and Fig 3). These distinctions persisted across Self Etch and Total Etch modes, further emphasizing the differential impact of treatments.

Figure 2: Control group

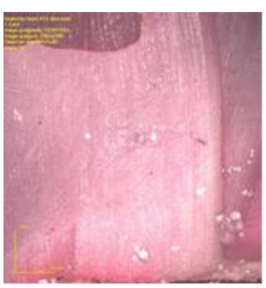

Figure 3: 10% Bromelain group

When considering the differences between Self Etch and Total Etch modes, distinct trends emerged. The Control group demonstrated no significant difference in nanoleakage between Total Etch mode and Self-Etch mode (Table 2). Conversely, the 10% Bromelain group displayed a significant reduction in nanoleakage levels in Total Etch mode (Table 3), establishing its adaptability and enhanced effectiveness under this condition (Fig 4). The 2% Ficin group consistently exhibited high nanoleakage levels in both modes (Table 3 & 4), with a slight reduction observed in Total Etch mode, indicating minimal influence of the etching technique on its performance (Fig 5).

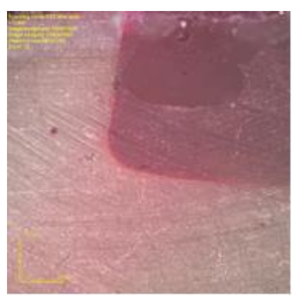

Figure 4: Total Etch mode 10% Bromelain group

Figure 5: Total Etch mode 2% Ficin group

Table 3: Comparison of mean Nano-leakage (in nm) in Self Etch mode b/w 3 groups

| Groups | N | Mean | SD | Min | Max | p-value |
|---|---|---|---|---|---|---|
| Control | 10 | 1423.18 | 95.48 | 1227.2 | 1518.1 | <0.001* |
| 10% Bromelain | 10 | 1064.99 | 55.99 | 998.0 | 1156.8 | |
| 2% Ficin | 10 | 2265.11 | 124.21 | 2129.8 | 2472.3 | |

Table 4: Comparison of mean Nano-leakage (in nm) in Total Etch mode b/w 3 groups

| Groups | N | Mean | SD | Min | Max | p-value |
|---|---|---|---|---|---|---|
| Control | 10 | 1398.35 | 138.08 | 1198.9 | 1598.5 | <0.001* |
| 10% Bromelain | 10 | 382.81 | 68.05 | 238.4 | 456.0 | |
| 2% Ficin | 10 | 2122.86 | 108.82 | 1917.6 | 2290.3 | |

The overall ranking of groups, based on their nanoleakage values, reflected a consistent progression across both etching modes. The 10% Bromelain group showed the lowest values, followed by the Control group, and lastly the 2% Ficin group with the highest levels. These findings highlight the variability in outcomes depending on the treatment and etching method employed, while underscoring the distinct effects of these factors on nanoleakage levels. The comparative analysis provides valuable insights into the effectiveness of treatments, demonstrating the superior performance of the 10% Bromelain group across both etching modes and reaffirming the critical role of treatment selection in optimizing sealing properties.

## Discussion

Modern adhesive dentistry is undergoing major transformations in the last two decades with the development of user-friendly universal adhesive systems and universal composite resins. The concept of nanoleakage by Sano et al was studied using scanning electron microscopy (SEM). This study used confocal laser scanning microscopy (CLSM) because of its merits being the formation of high- resolution histo-tomographic images of the layers in humid samples below the surfaces. The SEM and TEM techniques causes the creation of artefacts and qualitative evaluation of the layer is difficult. Both these reasons can be eliminated by the use of CSLM technique.

Rhodamine B, a fluorescent dye was used as the penetration agent in the study. There are 13 different modifications of this dye. Most of them being water-soluble.[14] But in our study, the dye used was dissolved in 50% alcoholic solution because of the conditions of this study. Distilled water was used as the solvent.

The dye was quantified based on the methods established for microleakage in the literature.[15] The penetrations in this study was taken from restoration margins till a certain depth of the cavity wall. The diffusion behaviour of the dye in a conical shape was noted during the 24 hour exposure time.

The results of this study showed that nanoleakage was least with 10% Bromelain group compared to 2% Ficin groups and the control groups. In this present study, bromelain enzyme performed better which could be because of reduced nanoleakage as shown by the previous study by Dayem et al.[16] It has better effectiveness in removing unsupported collagen matrix as compared to NaOCl, and lower nanoleakage is seen. This could be because of the depletion of collagen from the surface of acid-etched dentin resulting in increased permeability of dentin substrate due to the enlargement of dentinal tubules near the outer dentin surface.[17] This enhances the spreading and diffusing of adhesive monomers through dentin.[18] The surface energy of the dentin is improved, because the hydroxyapatite has a high surface energy substrate while collagen has a low energy surface, and this leads to enhanced diffusion of adhesive monomers through dentin. Also, the dentin is very porous and rough with many lateral branches of tubules which are detectable in main tubules, which may contribute to the increase in the spreading of adhesive monomers through dentin. [19]

Ficin is a proteolytic enzyme derived from fig latex and belongs to the class of cysteine endopeptidases. It contains chemicals that might help break down proteins and has various medical uses like to kill intestinal worms or indigestion. In dentistry it has been evaluated for its anti-biofilm property. The effect of Ficin on biofilms formed by one or multiple species has been studied and the proteolytic destruction of proteins can serve as a tool for biofilm growth prevention.[20] But it has not been evaluated for deproteinising agent and more studies are needed to prove its efficiency.

The results of this study also showed that single bond universal in the self-etch technique recorded significantly higher nanoleakage mean values than the total-etch technique. The results are in agreement with Munoz et al study. This may be due to the presence of the smear layer which constitutes a true physical barrier and makes it extremely difficult for the bonding and hybrid layer formation to be fully integrated with the dentine. After preliminary etching with phosphoric acid in etch & rinse approach, the smear layer is removed and superficial dentine is demineralized. This increases impregnation by the adhesive, allowing the creation of a well impregnated hybrid layer.[21]

It can be speculated that the modes of adhesive investigated in this study, can obtain a reliable adhesion. However, silver ion accumulations were noted in all the groups, causing nanoleakage. The study indicated that none of the deproteinising agent could prevent nanoleakage completely. Based on CLSM images, 10% bromelain showed least nanoleakage.

The nanoleakage phenomenon is shown to influence the marginal discoloration, recurrent caries, post- operative pain symptoms, and a decrease of the longevity of the composite restoration. Further studies are needed to develop adhesive systems minimizing nanoleakage and the use of deproteinising agent prior to bonding in order to optimize dentinal bonding.

**Conclusion**

Within the limitations of this study, it can be concluded that the application of 10% bromelain as a deproteinizing agent after acid etching significantly reduced nanoleakage compared to both the control and 2% ficin groups. Although none of the agents completely eliminated nanoleakage, bromelain demonstrated superior performance in enhancing the adhesive interface.

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