Original Article

Effect of Preetching on Microleakage with All-In-One Adhesives Using Calcium-Based Desensitizers: An *In vitro* Study

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Abstract

Objectives: The aim of this study was to evaluate the effect of calcium-based desensitizers on the microleakage with and without preetching enamel in Class V cavities restored with all-in-one adhesives. **Materials and Methods:** Class V cavities were prepared on the buccal surfaces of 100 extracted human premolars. A total of 100 box-shaped cavities were divided into five groups ($n = 20$). Group 1 – no desensitizer was applied and Groups 2 and 4 – desensitizer (CCP-ACP and Novamin) was applied, respectively, followed by the application of G-Bond and restored with composite restoration. Groups 3 and 5 are same as Group 2 and 4, but preetching of enamel was done for 3 s after desensitizers application. The teeth were thermocycled and the specimens were examined for microleakage using methylene blue as a marker. The teeth were sectioned buccolingually and evaluated for microleakage under stereomicroscope and the scores obtained were analyzed with Kruskal–Wallis and Mann–Whitney tests. **Results:** Statistically significant difference existed between Groups 2 and 3 ($P < 0.05$). There was also statistically significant difference between Groups 4 and 5 ($P < 0.05$). Groups 3 and 5 showed significantly less microleakage ($P < 0.05$). No statistically significant difference in microleakage values was observed between the two desensitizers CCP-ACP and Novamin. **Conclusion:** The application of calcium-containing desensitizers with selective etch enamel technique in all-in-one adhesives could be considered an advisable procedure to minimize microleakage.

Keywords: *All-in-one adhesive, CCP-ACP, Novamin, preetching of enamel*

Introduction

Dentinal hypersensitivity (DS) is a common oral complaint in daily dental clinics which probably affects 8%–35% of the population.[1] DS can be defined as pain arising from exposed dentin typically in response to chemical, thermal, tactile, or osmotic stimuli, which cannot be explained as arising from any other form of dental defect or pathology.[2] It occurs as a result of wear, caries, noncarious cervical lesions or after dental procedures such as cavity preparation or reduction of vital abutment teeth.[3,4] According to well-accepted hydrodynamic theory, stimulus to the exposed dentin surfaces disturbs the fluid movement within the dentinal tubules and consequently stimulates mechanoreceptors at pulp-dentin border leading to pain sensation.[5]

Cervical restorations are ever challenging because of difficulties in moisture control, caries access, and proximity to the gingival margin. In these restorations, microleakage acts as a seed for leakage of bacteria and oral fluids to invade the resin-dentin border causing sensitivity.[6] Studies demonstrated that different types of tubular occlusion agents can significantly reduce the fluid filtration across dentin and decrease the pain.[7] Currently, calcium phosphate-containing desensitizers have evoked considerable interest due to their biocompatible property, their outstanding characteristic in dentinal tubule occlusion by forming dentin-like minerals and favorable reduction in dentin permeability in the oral environment.[8,9]

Two calcium-based desensitizing pastes, casein phosphopeptide-amorphous calcium phosphate-containing paste (CPP-ACP, GC, Tokyo, Japan) and calcium sodium phosphosilicate-containing paste (Novamin, Smithkline Beecham Consumer Healthcare, Berkshire, UK), were used in this study.

CPP-ACP combination localizes in the form of nanoclusters and causes remineralization

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of enamel at a much faster rate, by maintaining high concentrations of calcium and phosphate ions on dentin surface.[10]

Novamin, a calcium-sodium phosphosilicate bioactive glass has been developed for the treatment of hypersensitivity by the physical occlusion of dentinal tubules, recent studies have demonstrated a potential for this material to prevent demineralization and/or aid in remineralization of tooth surfaces.[10]

Self-etch adhesives are very attractive for routine use in a busy daily practice and less possible to cause sensitivity. Shorter clinical application time and less incidence of postoperative sensitivity have made self-etch adhesive systems, a promising approach when compared to the etch and rinse systems.[11,12] It has been shown that morphology of dentin acid–base-resistant zone (ABRZ) was highly adhesive in self-etch adhesive systems than etch and rinse systems.[13] Besides micromechanical interlocking through hybridization, specific functional monomers in mild self-etch adhesives were shown to interact chemically with the Ca^{2+} of the residual hydroxyapatite that remains available within the submicron hybrid layer.[14] Likewise, the functional monomers may interact with Ca^{2+} contained in the desensitizer modified smear layer, potentially resulting in additional chemical bonding.[10]

In accordance to previous studies, G-bond used in the study is a mild self-etch adhesive, protects more vulnerable bond to dentin against degradation and generally perform not that favorably at enamel margins of a composite restoration.[15] Attempts to overcome this, have introduced selective enamel approach, which has proven to show good microtensile bond strength in the recent studies.[16,17]

In the literature, there are only few studies about the use of calcium-containing desensitizers evaluating the microleakage when used along with self-etch adhesives, but no up-to-date publication has been found that reports the microleakage of CPP-ACP and Novamin desensitizers using selective enamel etch technique. Hence, the current study aimed to assess the microleakage in Class V cavities restored with a mild self-etch adhesive (G-bond) using selective etch technique in combination with two calcium-containing desensitizers (CCP-ACP and Novamin).

Materials and Methods

One hundred carious-free human premolars with intact enamel surfaces were used. After extraction, to remove tissue remnants, they were hand scaled and stored in 0.5% aqueous chloramine T solution under refrigeration until use. Class V cavities were prepared 1 mm above the cementoenamel junction on the buccal surfaces of each tooth, using a rounded cylinder diamond bur (Jota Ag Rotary Instruments, Ruthi, Switzerland, ISO no. 806,314,140,534,012) at high speed with air/water spray. The cavity preparations were standardized with a width of 5 mm, a depth of 2 mm, and a height of 3 mm. These distances were measured with a digital caliper (Digital Slide Caliper, Tchibo GmbH, Hamburg, Germany).

Application of desensitizer, preetching the enamel (Group 3 and 5), and bonding

The materials, manufacturers, compositions, and application modes used in the present study are listed in Table 1. Two calcium-containing desensitizing pastes, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP, GC, Tokyo, Japan), and calcium-sodium phosphosilicate (Novamin, Smithkline Beecham Consumer Healthcare, Berkshire, UK), were used in this study. Prepared teeth were randomly divided into five groups as follows (n = 20 each group):

- Group 1 (Control): Cavities were not treated with any desensitizer, preparations were restored with G-bond (all-in-one adhesive) and Filtek Z350 XT shade A2, according to manufacturer's instructions
- Group 2: Cavities were treated with CPP-ACP desensitizer as described in Table 1, then G-Bond was applied and restored with Filtek Z350 XT
- Group 3: Cavities were treated with CPP-ACP desensitizer and preetched the enamel for 3 s followed by application of G-bond and composite restoration with Filtek Z350XT
- Group 4: Restored same as Group 2, but the desensitizer used was Novamin
- Group 5: same as Group 3, but the desensitizer used was Novamin.

The same operator performed all cavity preparations and restorations. All teeth were finished and polished using composite finishing bur 7901and Sof-Lex disks.

All areas of the teeth were covered with two coats of acid-resistant nail polish, except the restoration and 1 mm rim around it. The apices were sealed with sticky wax and the teeth were thermocycled. Thermocycling involved submerging the teeth for 10 s in water baths maintained between 5 and 55°C for 500 cycles, then immersing them in 0.5% methylene blue dye solution at room temperature for 24 h, later removing them, and rinsing them under running water.

Each sample was sectioned longitudinally in the middle in the buccolingual direction, using a diamond disc (Diamond Wafering Blade; Buehler, IL, USA) with a precision cutting machine (Isomet 1000, Buehler) under water cooling to attain two equal halves. The degree of marginal leakage was evaluated based on the penetration of the dye stain from the cavosurface margins to the base of the cavity preparation. Each specimen was viewed under a stereomicroscope (Olympus SZ61, Munster, Germany) at 30X magnification.

Two-independent precalibrated investigators independently examined the leakage scores. The investigators gave

Table 1: The materials, manufacturers, compositions, and application modes

Material	Manufacturer	Composition	Application mode
Etchant	3M ESPE, St. Paul, MN, USA	35% phosphoric acid solution, water, synthetic amorphous silica, polyethylene glycol, and aluminum oxide	Applied, left in place for 3 s, rinsed for 30 s with water spray
CPP-ACP-based desensitizer (tooth mousse)	GC, Tokyo, Japan	Glycerol, 5%-10% CPP-ACP, pure water, zinc oxide, CMC-Na, xylitol, D-sorbitol, silicon dioxide, phosphoric acid, titanium dioxide, guar gum, sodium saccharin, ethyl-p-hydroxybenzoate, magnesium oxide, propylene glycol, butyl-p-hydroxybenzoate, propyl-p-hydroxybenzoate	Applied with an applicator brush for 60 s, left for 3 min. Then, the specimens were rinsed with water spray for 30 s to remove loosely bound pastes on dentine surface
Novamin-based desensitizer (repair and protect)	Smithkline Beecham Consumer Healthcare, Berkshire, UK	Glycerin, PEG-8, silica, calcium-sodium phosphosilicate (Novamin), sodium monofluorophosphate, aroma, titanium dioxide, carbomer, potassium acesulfame, limonene	Applied with an applicator brush for 60 s, left for 3 min. Then, the specimens were rinsed with water spray for 30 s to remove loosely bound pastes on dentine surface
G-Bond	GC, Tokyo, Japan	4-MET, phosphoric monomer, UDMA, TEGDMA, stabilizer, photoinitiator, silica filler, acetone, water pH 2	Apply adhesive wait for 10 s air blow for 5 s light cure for 10 s
Filtek Z 350 XT	3M ESPE	Bis-GMA, UDMA, TEGDMA, Bis-EMA, particles of silica and zirconia/silane, BHT, photoinitiator system and pigments	Apply in increments of 2 mm light cure for 40 s in all directions

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate; Bis-GMA: Bisphenol A glycidylmethacylate; UDMA: Urethane dimethacrylate; TEGDMA: Triethylene glycol dimethacrylate; Bis-EMA: Bisphenol A ethoxylateddimethacrylate; BHT: Butyl hydroxyl toluene

leakage scores according to the depth of dye penetration. The degree of leakage was specified according to the following scoring system.[18-20]

1. No evidence of dye penetration at the tooth/restoration interface
2. Dye penetration along the interface to ≤half the depth of the cavity
3. Dye penetration to the full depth of the cavity
4. Dye penetration to the base of the cavity and beyond.

Results

Statistical analysis was performed utilizing the Kruskal–Wallis test followed by Mann–Whitney test. All the statistical tests were performed at a $P < 0.05$ level of significance. The mean microleakage values were presented in Tables 2-7. Stereomicroscopic images of all groups are shown in Figures 1-5, respectively. None of the groups showed the complete absence of microleakage. Among all the groups tested, control group has shown high microleakage scores ($P < 0.05$). There was a statistically significant difference between the Groups 2 and 3, while Group 3 showed less microleakage ($P < 0.00$). Among the Groups 4 and 5, Group 5 showed less microleakage. No statistically significant difference was observed between two desensitizers tested are presented in Tables 5-7, respectively.

Discussion

The present study investigated the effect of CCP-ACP and Novamin on microleakage, with and without preetching enamel (3 s only) of all-in-one adhesives. Our results showed that none of the groups in the study could completely eliminate the microleakage. Despite continuous improvement of adhesive systems, microleakage is still a major concern in restorative dentistry and deserves considerable study. Good marginal sealing the through use of appropriate desensitizers and adhesive systems can help to eliminate microleakage and reduce postoperative sensitivity.

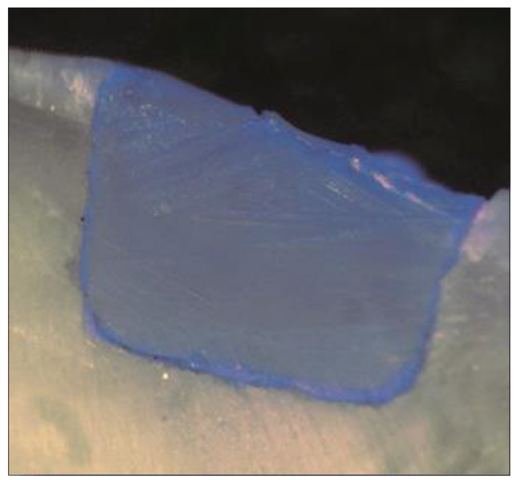

Figure 1: Group 1 (Control) were not treated with any desensitizer

Adhesive systems have also been substantially improved, employing material specific formulations, and simpler application techniques.[21] Single-step self-etch adhesives were chosen in the study as they do not remove the smear layer and the smear plugs, but only modify these structures, consequently the tubular orifices remain sealed by the plugs. It is believed that this mechanism of adhesion, that enables bonding without exposing the dentinal tubules, is responsible for eliminating microleakage and thus postoperative sensitivity.[22]

Scanning electronic microscope observation at dentin–adhesive interfaces reported the presence

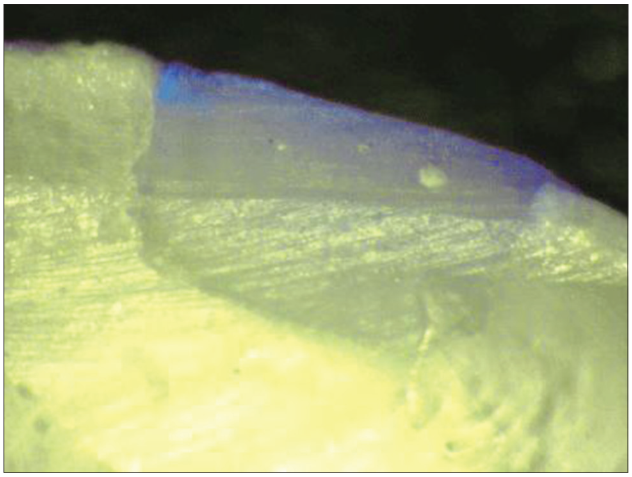

Figure 2: Group 2: Cavities were treated with casein phosphopeptide-amorphous calcium phosphate-containing paste desensitizer, then G-Bond was applied and restored with Filtek Z 350 XT

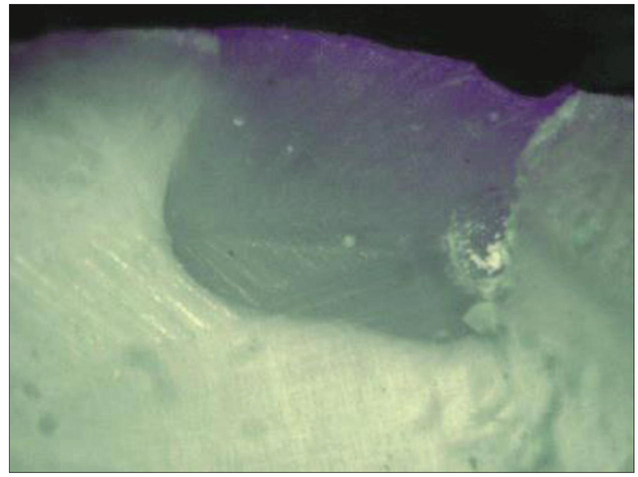

Figure 3: Group 3: Cavities were treated with casein phosphopeptide-amorphous calcium phosphate-containing paste desensitizer and preetched the enamel for 3 s followed by application of G-Bond and composite restoration with Filtek Z350XT

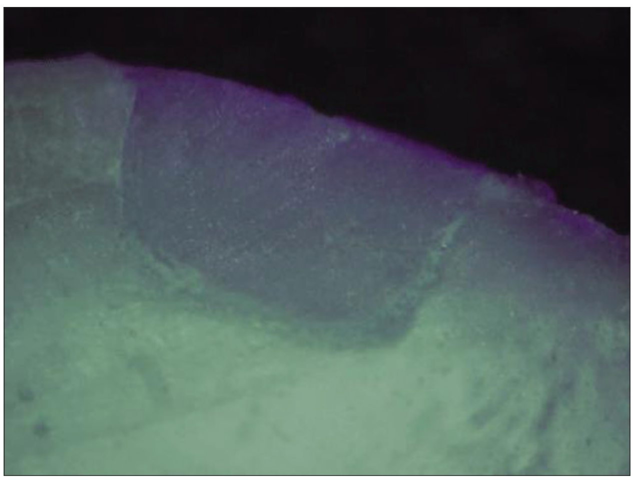

Figure 4: Group 4: Restored same as Group 2, but the desensitizer used was Novamin

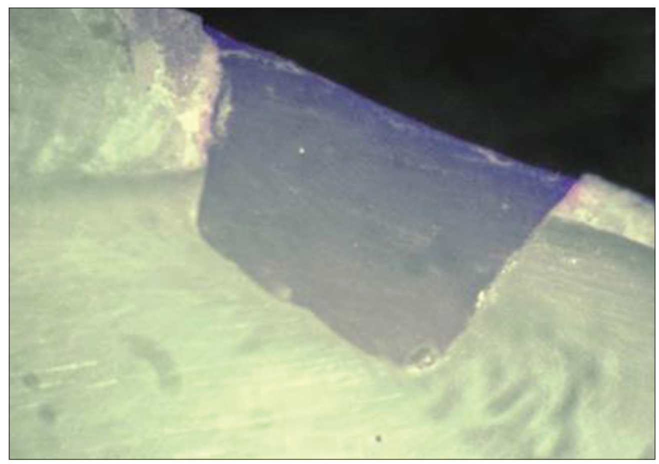

Figure 5: Group 5: Same as Group 3, but the desensitizer used was Novamin

of an ABRZ beneath the hybrid layer after acid–base challenge in all-in-one adhesives was better in comparison to total-etch adhesives.[13] G-Bond used in this study contained the functional monomers 4-methacryloyloxyethyltrimelliticacid (4-MET) which have been reported to contribute to resin-dentin bonding. Potential interaction of calcium contained in the desensitizers and 4-MET results in the production of additional chemical bond. Pei *et al*., 2013 reported that microtensile bond strength of G-Bond was not influenced by pretreatment with CCP-ACP-containing paste. Although the single-step self-etch adhesives are easy to use in the present day dental practice, there are shortcomings and could not completely eliminate microleakage.[15] It was shown that when using all-in-one adhesives, the margins of restorations in enamel were worse than the margins of restorations where phosphoric acid was used.[19]

Phosphoric acid preetching of enamel attacks the hydroxyapatite crystals, partially eroding them and creating a porous and retentive structure on the surface.[23] Therefore, the acidic functional monomers may be able to bond chemically to preetched enamel as effectively as to dentin. Therefore, these results indicate that simplified all-in-one adhesive systems (G-Bond) need preetching of the margins with phosphoric acid for an effective seal.[19] Hence, an attempt was made on preetching of enamel (3 s) as the previous studies suggest that reduced phosphoric acid preetching times do not impair the fatigue bond strength of universal adhesives.[24,25]

Our study results have shown that there was statistically significant reduction in microleakage in preetched Groups (3 and 5) with either of the desensitizers (CCP-ACP and Novamin) which might be because of preetching of enamel (3 s) with phosphoric acid attacks the hydroxyapatite crystals, partially eroding them, and creating a porous and retentive structure on the surface.[23] Therefore, the acidic functional monomers may be able to bond chemically to preetched enamel as effectively as to dentin.[24]

Table 2: Intergroup comparison in self-etch group using Kruskal-Wallis test and significant difference in microleakage values with respect to control group were observed

Self-etch groups	Group	Mean	SD	*P*
1.00	Control group	2.6000	0.50262	0.00
2.00	CCP-ACP (without preetching)	2.4000	0.50262	
3.00	CCP-ACP (with preetching)	1.4000	0.82078	
4.00	Novamin (without preetching)	2.0000	0.79472	
5.00	Novamin (with preetching)	0.9000	0.30779	

$P<0.05$, statistically significant. SD: Standard deviation

Table 3: On intergroup comparison using Mann-Whitney test, there was significant difference in microleakage scores with Group 3 showing better results

Groups	Mean	SD	*P*
Group 2	1.9000	0.84124	0.00
Group 3	2.5000	0.50637	

$P<0.05$, statistically significant. SD: Standard deviation

Table 4: On intergroup comparison using Mann-Whitney test, there was significant difference in microleakage scores with Group 5 showing better results

Groups	Mean	SD	*P*
Group 4	1.4500	0.81492	0.00
Group 5	4.5000	0.50637	

$P<0.05$, statistically significant. SD: Standard deviation

Table 5: On intergroup comparison using Mann-Whitney test, there was no statistically significant difference in microleakage scores between Groups 2 and 4

Groups	Mean	SD	*P*
Group 2	1.5010	0.541242	1.01
Group 4	3.5000	1.01027	

$P>0.05$, statistically no significant difference. SD: Standard deviation

Table 6: On intergroup comparison using Mann-Whitney test, there was no significant difference in microleakage scores between Groups 3 and 5

Groups	Mean	SD	*P*
Group 3	0.975	1.026	0.621
Group 5	0.925	0.504	

$P>0.05$, statistically no significant difference. SD: Standard deviation

Table 7: On intergroup comparison using Kruskal-Wallis test, there was no significant difference in microleakage scores on comparison of Groups 1, 2, and 4

Groups	Mean	SD	*P*
Group 1	2.956	2.500	0.512
Group 2	1.935	1.495	
Group 4	1.962	1.465	

$P>0.05$, statistically no significant difference. SD: Standard deviation

Results of the present study have shown higher microleakage scores in Groups 2 and 4 which could be because hydroxyapatite crystals within enamel are considerably larger than those within dentin which interfere with acidic functional monomers further affecting the chemical bonding when compared to dentin.

This is important for clinicians who are concerned about the additional time required for using phosphoric acid preetching with all-in-one adhesives.[24]

Yang *et al*., 2018 have evaluated the tubular occlusion of CCP-ACP and Novamin, the CPP-ACP-treated dentine presented the formation of numerous crystals on the dentine surface, with occluded tubules. The square average roughness values presented by atomic force microscopy values were increased which indicating more wet surface and lower contact angles, which favors of mechanical interlocking and adhesion, hence they were chosen in this study.

In terms of functional mechanisms, CPP could stabilize ACP on the dentine surface after CPP-ACP treatment; Ca^{2+} and PO_4^{3-} maintained at high concentrations in the hybrid layer, thereby forming an ion osmotic gradient and promoting the remineralization of the hybrid layer and demineralized dentine.[26] In Novamin-treated dentin, Na^+ exchanged with H+ or H_3O+ and the pH increased, which resulted in the continuous release of Ca^{2+} and PO_4^{3-} from Novamin to form microcombination with the original hydroxyapatite crystals for reinforcing the hybrid layer, which is in accordance to previous studies.[27-29] This speculations can be tested by further research *in vivo* on a long-term basis. *In vitro* and clinical studies of different desensitizers with selective enamel-etch technique in all-in-one adhesives have to be proven for better marginal sealing.

Conclusion

Application of CCP-ACP or Novamin without pre-etching could not eliminate microleakage.

Desensitizers application followed by preetching for 3 s on enamel have significantly reduced microleakage than unpreetched groups.

This new generation all-in-one adhesives with minimal preetching time (3 s) on enamel may be a preferable method for improving the marginal sealing of cervical restoration.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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