

# COMPARATIVE EVALUATION OF THE INFLUENCE OF PRE-TREATMENT WITH CPP-ACP AND NOVAMIN ON DENTINAL SHEAR BOND STRENGTH WITH COMPOSITE: AN IN VITRO STUDY

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## ABSTRACT

**Aim:** The purpose of this study was to evaluate the effects of application of CPP-ACP and NovaMin pastes on the cut dentin surface with and without retention of smear layer, on bond strength of the resin dentin interface, with an all-in-one adhesive.

**Materials & Method:** Dentin surfaces of sixty extracted human permanent posterior teeth were exposed. The samples were then divided into two groups of 30 each. In one-half the smear layer was retained, while in the other half it was removed. The samples were further divided into 3 subgroups: the untreated controls, CPP-ACP and NovaMin pastes treated; applied for 5 minutes each. Further a self-etch all-in-one adhesive was applied and hybrid resin composite cylinders were cured. The shear bond strength was measured under a universal testing machine.

**Results:** One-way ANOVA and Post-Hoc Tests (Tukey's–HSD) tests were used. The smear layer retained group showed better results than the group with no smear layer. Further the group with smear layer retained and NovaMin paste application showed a statistically significant increase in bond strength.

**Conclusion:** Smear layer retained group and pretreatment with Novamin showed the highest shear bond strength.

**Key words:** CPP-ACP (Tooth Mousse), NovaMin (Vantej), Shear Bond Strength, Universal Testing Machine.

## Introduction

The search for an ideal aesthetic material for restoring teeth has resulted in significant improvements in both aesthetic materials and techniques for using them. Adhesive materials that have stronger bonds further simplify the restorative techniques.<sup>1</sup> In dentistry, the adherent is the substrate to which the adhesive— enamel and dentin, rarely cementum - is applied.<sup>2</sup> As first discussed by Buonocore, bonding to dentin is difficult since, it contains a significant amount of water and organic material (mainly Type I collagen).<sup>2</sup>

Bonding of the tooth colored restorative materials since the introduction of sixth generation adhesives has become easier and faster. With the advent of seventh generation adhesives which are truly referred to as “one-bottle systems” the conditioning, priming and application of adhesive resins have been combined.<sup>1</sup> The advantages of one-step self-etch adhesives are that they have the most time-efficient application procedure, have less sensitivity to diverse dentin-wetness conditions and simultaneous demineralization and resin infiltration.<sup>1</sup> The bonding to dentin of self-etch adhesives occurs by virtue of mild etching, micromechanical bonding which is achieved with a hybrid layer and resin tag formation and also due to chemical interaction between the calcium of the partially demineralized substrate (i.e. dentin and its smear layer) and the ingredients of the adhesive i.e. 10-methacryloyloxy decyldihydrogen phosphate (10-MDP)<sup>2</sup> and 4-methacryloxyethyl trimellitic acid (4-MET).<sup>1</sup>

Whenever dentine is cut using hand or rotary instruments, a layer called as the ‘smear layer’ is formed comprising the hydroxyapatite, altered collagen matrix, saliva, blood and numerous microorganisms.<sup>3</sup> It is of 0.5-2  $\mu\text{m}$  layer

thickness.<sup>4</sup> It seals the openings of dentinal tubules, called as ‘smear plugs’ which extend to a depth of 1-10  $\mu\text{m}^2$  and covers the intertubular and peritubular dentin. The question of keeping or removing the smear layer remains controversial.<sup>5</sup>

For effective removal of smear layer, it is generally recommended to use ethylenediamine tetra acetic-acid (EDTA) which is the most effective chelating agent with prominent lubricant properties. It removes the smear layer in <1 minute if the fluid is able to reach the root canal surface wall.<sup>6</sup>

The adhesive techniques that require removal of smear layer are associated with postoperative sensitivity than systems that leave the smear layer in place. Thus, the self-etch bonding agents are considered to be an alternative as they penetrate the smear layer and incorporate into the bonding layer.<sup>2</sup>

Dentin may become exposed due to caries or due to the development of non-carious lesions. Non-cavitated as well as cavitated lesions extending upto the dentinoenamel junction can be arrested by remineralizing agents such as fluoride-delivery methods or other bioactive agents based on milk products. Also, non-carious cervical lesions are often associated with dentine hypersensitivity. Again, management of hypersensitive dentin involves remineralization or occlusion of patent dentinal tubules.<sup>4</sup>

A novel calcium phosphate remineralization technology has been developed based on casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [Recaldent™].<sup>7</sup> It inhibits enamel and dentin demineralization and promotes remineralization in several independent studies. Casein phosphopeptides derived from bovine milk contains 30 mM calcium (68%) and 22 mM inorganic

phosphate (47%) that have been reported to bind amorphous calcium phosphate forming nanoclusters of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), thereby stabilizing calcium phosphates in solution. CPP structure containing the active sequence – Ser(P)-Ser(P)-Ser(P)-Glu-Glu- has a remarkable ability to stabilize calcium and phosphate as nanoclusters of ions in metastable solution. Although the calcium, phosphate, and fluoride ions are stabilized by the CPP from promoting dental calculus, the ions are freely available to diffuse down concentration gradients into enamel subsurface lesions, thereby effectively promoting remineralization *in vivo*.<sup>7</sup>

Another remineralization technique is a class of compounds called bioactive glass that has been available since late 1960s. NovaMin<sup>®</sup>, is the trade name for a calcium sodium phosphosilicate compound which has been recently launched as a remineralizing agent.<sup>8</sup> The mode of action of this material results from interactions with saliva. The active ingredient is a calcium sodium phosphosilicate that reacts when exposed to aqueous media and provides calcium and phosphate ions that form a hydroxy-carbonate apatite (HCA) with time that is structurally and chemically similar to the natural tooth mineral.<sup>5</sup>

Though the self-etching adhesives have decreased the technique sensitivity and have proven to be time saving, they have not yet been made comparable in bonding strength to the total etch adhesives. There have been many methods and novel approaches devised, to try and increase the bond strength of the self-etch adhesives. Hence forth, the effect of increase of mineral content of dentin or smear layer by either CPP-ACP or NovaMin<sup>®</sup> on bonding efficiency of the all-in-one adhesives is an avenue worth investigating as numerous studies demonstrating the potential of both these materials in the areas of caries prevention have proven their efficacy in remineralization.

The presence and quality of the smear layer affects bonding of self-etching and priming adhesives. So, if the mineral content of the smear layer can be increased by preconditioning then the bond strength of even the mild self-etch adhesive should drastically increase.<sup>4</sup>

Thus, the purpose of this study was to evaluate and compare the shear bond strengths of resin-dentine bond after surface pretreatment of the cut dentin surface (with or without the retention of smear layer), with Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) and Calcium Sodium Phosphosilicate (NovaMin)<sup>®</sup> pastes.

## Materials and Method

### Sample Selection and Preparation-

A total of sixty freshly extracted human permanent non-carious molars were selected for the study. These were collected as per the norms laid down by the institutional ethical committee. Teeth with any caries, cracks, abrasions, attrition and restorations were excluded from the study.

Any extrinsic stains or calculus deposits on teeth were cleaned using an ultrasonic scaler. They were then stored in normal saline with 1% thymol at room temperature, till the study was conducted. Collection, storage, sterilization and handling of extracted teeth used in the study followed the Occupational Safety and Health Administration (OSHA) and the Centre for Disease control and prevention (CDC) recommendations and guidelines.

The stored teeth were retrieved from normal saline and depth holes measuring 1.5 mm were drilled in the deepest part of central fossa of each tooth with the help of a round diamond bur. This was done to standardize the depth of dentin as it also affects the dentin bond strength. All the sixty samples were then trimmed with a diamond disk to expose a flat dentinal surface. This was followed by manual polishing of the dentinal surface with 400, 600 and 1000 grit silicon carbide papers. The samples were embedded in an auto-polymerizing acrylic resin in the aluminium moulds. The specimens were placed perpendicular to the acrylic resin surface. The samples were then washed with water, dried with air from an oil-free air source.<sup>5</sup>

Further the samples were randomly divided into two main groups. In Group A, the smear layer was left in place for the treatment/bonding process, while in Group B the smear layer was removed by applying 17% EDTA solution for 60 seconds and then gentle rinsing for 15 seconds using an air-water spray.

### Remineralizing pastes application-

In each group, the samples were further divided into 3 subgroups of 10 each. The untreated controls (IA, IB), Tooth Mousse (CPP-ACP) treated (IIA, IIB) and Vantej (Novamin) treated (IIIA, IIIB). The samples were then placed in a tray. Tooth Mousse and Vantej each was applied for 5 minutes to just cover the surface of dentin.

### Self-Etch Adhesive application-

An adhesive tape with a punch hole of diameter 3mm was applied on the flat dentinal surface of each mounted tooth to delineate the area for bonding. The self-etch adhesive, Adper<sup>™</sup> Easy One (3M ESPE) was applied thereafter according to the manufacturer's instructions. It was applied on the uncovered dentin surface with a teflon coated placing instrument and fibre applicator tips, left undisturbed for 10s and thinned with a mild air-blast for 5s. It was cured in place using a light-emitting diode curing light of 800mW/cm<sup>2</sup> for 20s.

### Composite Cylinder preparation and bonding-

Transparent plastic tubes of internal diameter 2mm and height 3mm with thickness 0.5mm were pre-cut and placed perpendicular to dentinal surface on the bonding agent. A hybrid resin composite (Filtek<sup>™</sup> Z350 XT, Body Shade A1) was loaded into the pre-cut tubes and bonded to the adhesive by light curing for 20s. The tubes were then removed. The bonded specimens were finally placed in distilled water at room temperature for 24h.

**Shear Bond Strength Test-**

Twenty-four hours after bonding, the samples were removed and subjected to shear bond strength test using universal testing machine (Instron, ADMET, Enkay Enterprises, New Delhi) using the corresponding computer software. The specimens were seated such that the upper surfaces were flush with the edge of the jig, while a straight knife-edge rod was applied at the tooth restoration interface at a cross-head speed of 0.5mm/min. Load was applied until restoration failure occurred. The load at failure was converted to shear bond strength evaluation (MPa) by dividing the load by surface area of the specimen.

**Statistical Analysis**

The obtained data was subjected to statistical analysis using parametric tests at a significance level of  $p = 0.05$ . Mean and Standard deviations were calculated for each group. One-way ANOVA and Post-Hoc Tests (Tukey’s–HSD) tests were used.

**Results**

In this study, in Group A the smear layer was not removed, whereas Group B the smear layer was removed with 17% EDTA for 60 seconds. Each group was further divided into six subgroups: Subgroup IA, IIA, IIIA– Assessed without smear layer removal and Subgroup IB, IIB, IIIB – Assessed after smear layer was removed.

**I. Comparative evaluation of shear bond strength of different groups without removal of smear layer:**

Table 1 shows mean shear bond strength of different groups without removal of smear layer. In Group IA, shear bond strength of samples ranged from 4.27 to 20.13 MPa with a mean value of  $13.72 \pm 5.68$  MPa. In Group IIA, shear bond strength of samples ranged from 7.30 to 17.27 MPa with a mean value of  $11.27 \pm 3.62$  MPa. In Group IIIA, shear bond strength of samples ranged from 12.70 to 32.10 MPa with a mean value of  $20.91 \pm 5.48$  MPa. Minimum mean shear bond strength was observed for Group IIA and maximum for Group IIIA.

SN	Group	No. of Sample	Mean	SD	Range	
					Min	Max
1.	IA	10	13.72	5.68	4.27	20.13
2.	II A	10	11.27	3.62	7.30	17.27
3.	III A	10	20.91	5.48	12.70	32.10

Table 1: Comparative evaluation of shear bond strength (SBS) of different groups with smear layer retained

Table 2 shows a significant intergroup difference in mean shear bond strength ( $F=9.999$ ;  $p=0.001$ ).

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	502.468	2	251.234	9.999	0.001
Within Groups	678.372	27	24.125		
Total	1180.840	29			

Table 2: Analysis of variance for Mean Shear Bond Strength in different groups with smear layer

The order of mean shear bond strength in different groups without smear layer removal was:

**Group IIIA > Group IA  $\approx$  Group IIA**

**II. Comparative evaluation of shear bond strength of different groups after removal of smear layer (One-Way ANOVA):**

Table 3 shows mean shear bond strength of different groups after removal of smear layer. In Group IB, shear bond strength of samples ranged from 8.57 to 33.20 MPa with a mean value of  $18.78 \pm 7.07$  MPa. In Group IIB, shear bond strength of samples ranged from 1.77 to 26.63 MPa with a mean value of  $14.76 \pm 6.71$  MPa. In Group IIIB, shear bond strength of samples ranged from 9.47 to 24.47 MPa with a mean value of  $15.04 \pm 5.18$  MPa. Minimum mean shear bond strength was observed for Group IIB and maximum for Group IB.

SN	Group	No. of Sample	Mean	SD	Range	
					Min	Max
1.	I B	10	18.78	7.07	8.57	33.20
2.	II B	10	14.76	6.71	1.77	26.63
3.	III B	10	15.04	5.18	9.47	24.47

Table 3: Mean Shear Bond Strength of Different Groups after removal of smear layer

Table 4 shows analysis of variance for mean shear bond strength in different groups. Table 4 thereafter show no significant intergroup difference in mean shear bond strength ( $F=1.237$ ;  $p=0.306$ ). It was observed that all the three groups had shear bond strength values of similar order showing an overlap of interquartile ranges. It was observed that there both groups IIB and IIIB had some extreme values.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	100.410	2	50.205	1.237	0.306
Within Groups	1095.448	27	40.572		
Total	1195.858	29			

Table 4: Analysis of variance for Mean Shear Bond Strength in different groups after removal of smear layer

The order of mean shear bond strength in different groups after smear layer removal was:

**Group IB  $\approx$  Group IIB  $\approx$  Group IIIB**

**Discussion**

Non-carious cervical lesions are more common on buccal and lingual surfaces of posterior teeth; are associated with more open and wider dentinal tubules and have heterogenous, but thinner smear layers.<sup>4</sup> It is due to the tubular structure with the presence of odontoblastic processes, the moist condition due to the presence of dentinal fluid, intratubular pressure and permeability of dentin, that reliable and durable resin-dentin bonds remains a challenge till date. Adebayo OA *et al*<sup>4</sup> reported that the tubule arrangement is either parallel/oblique to the surface,

but as we go deeper the tubular arrangement becomes more perpendicular.

Owens *et al*<sup>8</sup> reported that the perfect adhesion to tooth structure is the primary objective. On all the samples an all-in-adhesive Adper™ Easy One was applied, a recently introduced HEMA-rich adhesive (2-hydroxymethyl methacrylate). Manufacturers claim it has improved shelf-life and does not require refrigeration due to the presence of a co-solvent ethanol. It also stated that the hydrophilicity of HEMA (2-hydroxymethyl methacrylate) makes it an excellent adhesion promoting monomer and by enhancing wetting of dentin, HEMA significantly improves bond strength. The HEMA rich and ethanol-water-based self-etch adhesives are the “golden standard” in terms of adhesion durability. Gregoire *et al*<sup>9</sup> reported that hydroxymethylmethacrylate (HEMA) are widely used monomers which also act as a solvent and prevent hydrophilic and hydrophobic phase separations and are present in the self-etch adhesives with solvents to increase bonding effectiveness.

The presence and quality of smear layer affects bonding of self-etching and priming adhesives and may reduce bond strengths. This study analyzed the effect of smear layer retained and removed on shear bond strength of dentine at resin-dentine interface. Bonding was carried out to dentin with or without prior smear layer removal prior to treatment with CPP-ACP and Novamin paste in an attempt to stimulate the clinical scenarios on exposed cervical dentin lesions. The removal of smear layer was carried out by applying SmearClear (EDTA) for 60 seconds. The methodology followed was in accordance with the study conducted by Madan *et al*.<sup>10</sup>

In this study the effect of Novamin application with smear layer retained was found to have a statistically significant increase in shear bond strength. The calcium sodium phosphosilicate (NovaMin®) known as a bioactive glass in the class of highly biocompatible materials was originally developed as a bone-regenerative material and so is now mostly used in oral health care.<sup>11</sup> Golpayegani *et al*<sup>12</sup> reported its use most commonly to prevent demineralization and/or aid in remineralization of tooth surfaces. The composition of NovaMin® comprises of 45% SiO<sub>2</sub>, 24.5% Na<sub>2</sub>O, 24.5% CaO, 6% P<sub>2</sub>O<sub>5</sub>.

They also reported that the mode of action of this material results from interactions with aqueous solutions. In aqueous environments, such as saliva, sodium ions (Na<sup>+</sup>) in calcium sodium phosphosilicate particles (CSP) immediately (within one minute) begin to exchange with hydrogen cations (H<sup>+</sup> or H<sub>3</sub>O<sup>+</sup>). This rapid exchange of ions allows calcium (Ca<sup>2+</sup>) and phosphate (PO<sub>4</sub><sup>3-</sup>) species to be released from the particle structure. A localized transient increase in pH occurs that facilitates the precipitation of calcium and phosphate from the particles and from saliva to form a calcium phosphate (Ca-P) layer on tooth surfaces.<sup>12</sup>

According to Golpayegani *et al*, on application of Novamin an increase in the mineral content of the smear layer is seen

on dentin. This remineralization of dentine also physically occludes dentinal tubules, which relieves hypersensitivity.<sup>12</sup> The combination of the residual CSP particles and the HCA layer results in the remineralization of either enamel or dentine and also physical occlusion of dentinal tubules, which relieves hypersensitivity.<sup>12</sup>

According to Schimidin PR *et al*<sup>13</sup> the calcium and phosphate ions were deposited on dentin from NovaMin for tubule occlusion. It also states that the remineralizing agents such as Novamin & CPP-ACP may provide stronger bond strengths for self-etch bonding agents for both enamel and dentin. Further Reynolds *et al*<sup>14</sup> reported that demineralizing & remineralizing cycles showed increased bond strengths for one-step self-etch adhesives.

In this study NovaMin (Vantej) paste was applied for 5 minutes on the cut dentin surfaces irrespective of the presence of smear layer. According to Wefel a generally linear relationship exists between exposure time and mineral deposition indicating the more the exposure time yields increased remineralization.<sup>7</sup>

Whereas the shear bond strength after the Tooth Mousse (CPP-ACP) paste pre-treatment for just 5 minutes with the smear layer retained in this study did not prove to be statistically significant. Since, it is a time dependent precipitation process and as per the manufacturer's instructions should be applied for several hours for at least a time period of one week.<sup>7</sup> This could be one of the probable reasons for the lack of effectiveness of CPP-ACP as compared to NovaMin on the shear bond strength.

Reynolds *et al* in 1980s in another study also identified the role of casein phosphopeptides (CPP) in the stabilization and localization of amorphous calcium phosphate (ACP) at the tooth surfaces. Thus this novel remineralization technology was developed and was reported to reduce enamel erosion, increase the hardness on enamel softened by cola drink and act as an anticariogenic paste for remineralizing the initial enamel caries lesions.<sup>6</sup>

According to Adebayo *et al* the bond failures for the all-in-one adhesive were found to be more pronounced within the hybrid layer following CPP-ACP treatment.<sup>4</sup> Zorba *et al*<sup>15</sup> reported that the shear bond strength values were statistically insignificant when the desensitizing agent, CPP-ACP was used to bond composite resin to dentine.

However in this study the control group with no pretreatment done, was found to be statistically insignificant when the smear layer was left in place. Tay *et al*<sup>16</sup> reported that the presence and quality of the smear layer affects the bonding of self-etching and priming adhesives thus reducing the bond strength. Oliveira *et al*<sup>17</sup> stated that the modification by the primer showed a significant inverse association between coarseness level and the tubule openness. Also, the thicker the smear layer the lesser the bond strength because of lack of open tubules and resin tag formation.

In this study the shear bond strength values for all the groups after the removal of smear layer were found to be statistically insignificant although they were marginally higher for the control group. Gilliam *et al*<sup>18</sup> stated that the patent tubules before treatment with Novamin and the occluded tubules after treatment showed a decrease in values because of the occluded dentinal tubules. Wefel<sup>7</sup> observed that a 2-minute brushing with the dentifrice every day for a period of 10 days resulted in an appropriate number of dentinal tubule occlusion.

As already mentioned, in the CPP-ACP group the absence of smear layer increased the bond strength values marginally but, could not stated as statistically significant. Adebayo *et al* who reported that the absence of smear layer with CPP-ACP pretreated dentin did not affect the shear bond strength.<sup>4</sup> Also he speculated that the closer apposition of the CPP-ACP paste to the dentine in this group resulted in a dentine surface that was harder to condition and prime for the all-in-one adhesive, due to enhanced mineral content of the dentine or the presence of residue from the paste.<sup>4</sup>

Similarly for the control group the removal of smear layer resulted in marginally higher shear bond strength values since there were more open tubules and more partially occluded tubules. Tay *et al* reported that the increased the bond strength was seen due to micromechanical retention, resin tag and hybrid layer formation.<sup>16</sup>

Finally when the mean shear bond strength values of the groups following treatment with CPP-ACP were compared, it was found that in the no smear layer group the mean values were higher but, not statistically significant than when the smear layer was left in place. Similarly, for the untreated controls were compared than in the no smear layer group the mean values were higher than when the smear layer was retained. On the contrary, for the group following treatment with NovaMin with the smear layer left in place was the highest than in the no smear layer group.

Under the limitations of this in vitro study, the removal of the smear layer had no significant effect on the shear bond strength at the resin dentin interface of all-in-one adhesive used. It can be stated that NovaMin with retained smear layer showed the highest shear bond strength so it could be used as a remineralizing agent. In other words, pretreatment can be done with NovaMin paste before restoring the tooth with composite resin.

Thus, studies regarding the shear bond strength measurement at the resin-dentine interface with pretreatment of dentinal surface with NovaMin are scarce. Further studies are required to confirm this preliminary report.

### Conclusion

- NovaMin paste treatment significantly increased the shear bond strength (SBS) when the smear layer was retained. Though it (SBS) remained unaffected when smear layer was removed.

- However, the SBS was not significantly affected when pretreated with CPP-ACP paste; either the smear layer was retained or removed.
- For the control group also the shear bond strength was not significant statistically, either the smear layer was retained or removed.

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