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Effect of dentin biomodifiers on shear bond strength and dentin-resin interface: An *in vitro* study

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Abstract:

Durable adhesion to dentin remains essential for the long-term success of adhesive restorations. Therefore, it is of interest to compare the effects of 6.5% grape seed extract (GSE), casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) and their combination on dentinal shear bond strength and the resin-dentin interface. Shear bond strength was measured using a universal testing machine and the resin-dentin interface was evaluated by scanning electron microscopy. Significant differences were observed among the groups ($p < 0.001$), with GSE showing the highest shear bond strength (3.39 MPa), greatest resin tag score and longest resin tags (70.20 μm). Thus, data shows the use of dentin biomodifiers, particularly GSE, to enhance dentin bonding and guide evidence-based adhesive pre-treatment strategies.

Keywords: Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP), grape seed extract (GSE), resin tags, scanning electron microscopy (SEM), shear bond strength (SBS)

Background:

The concept of adhesive dentistry has revolutionized contemporary restorative practice, enabling preparations that do not rely on conventional mechanical retention but instead adhere to tooth structure with minimal tissue loss [1]. This advance was made possible by Buonocore's introduction of the acid-etch technique, which converted the relatively unreactive enamel surface into one of high surface energy, allowing predictable bonding [2]. In contrast to enamel, adhesion to dentin remains challenging owing to the dynamic and heterogeneous nature of the substrate - its complex histological architecture, high tubular content and intrinsic moisture [3]. The strength of the resin-dentin bond depends on infiltration of the adhesive into the collagen matrix exposed by acid conditioning; the resulting interdiffusion zone, the hybrid layer, provides the primary micromechanical retention of the restoration [4]. However, adhesive penetration is generally shallower than the depth of demineralization and complete water displacement from the matrix is difficult, leaving exposed collagen that becomes susceptible to hydrolytic degradation by endogenous matrix metalloproteinase and cysteine cathepsins [5]. Most advances in adhesive dentistry have focused on bonding agents and techniques, with comparatively little attention to the contribution of collagen integrity to bond stability. The application of biocompatible cross-linking agents has therefore emerged as a strategy to enhance the mechanical properties of exposed dentinal collagen and to inhibit collagenolytic activity, thereby preserving the hybrid layer [6]. Among the available cross-linkers, Proanthocyanidins-rich grape seed extract (GSE) has attracted particular interest because of its ability to form additional inter- and intramolecular cross-links with collagen [7]. A complementary strategy is biomimetic remineralization of the water-filled collagen matrix using analogues of matrix proteins. Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) has been proposed to stimulate biomimetic remineralization of dentin collagen fibrils and may modify the morphology of the dentin-resin interface [8]. Therefore, it is of interest to evaluate and compare the dentinal shear bond strength and the quality of the resin-dentin interface following pre-treatment with 6.5% GSE and CPP-ACP, used alone and in combination.

Materials and Methods:

Sixty freshly extracted, non-carious human maxillary and mandibular premolars were collected following ethical guidelines and disinfected according to OSHA recommendations. Specimens were screened by digital radiography to exclude carious, restored, hyperplastic, fractured or endodontically treated teeth and stored in distilled water until use. All sixty samples were randomly assigned to four groups ($n = 15$) according to the surface treatment protocol: Group I - no treatment (control); Group II - 6.5% GSE; Group III - CPP-ACP; and Group IV - 6.5% GSE followed by CPP-ACP. From each group, ten samples were used for shear bond strength (SBS) testing and five for SEM analysis of the resin-dentin interface. A flat dentinal surface was prepared on each specimen by drilling a 1.5 mm depth hole at the centre of the crown with a small round diamond bur and trimming with a diamond disc under copious water irrigation; the exposed dentin was then sequentially polished with silicon carbide papers. Specimens were sectioned at the cemento-enamel junction and embedded perpendicular to their long axis in self-cure acrylic resin moulds. The flat dentinal surfaces of all sixty samples were acid-etched with 37% phosphoric acid for 15 seconds, rinsed and dried. Surface treatments were then applied as follows: in Group II, freshly prepared 6.5% GSE solution (In Life Group, India) was brushed onto the dentin, kept for 10 seconds, rinsed and dried; in Group III, CPP-ACP paste (Tooth Mousse MI Paste, GC Corporation, Tokyo, Japan) was brushed onto the etched surface, left for 3 minutes, rinsed and dried; in Group IV, the GSE solution was applied for 10 seconds, rinsed and dried, followed by CPP-ACP paste for 3 minutes, rinsed and dried. The 6.5% GSE solution was prepared by dissolving 6.5 g of GSE powder, measured on an analytical balance (Sartorius), in 100 mL of distilled water. Two coats of a fifth-generation bonding agent (Adper Single Bond, 3M ESPE) were then applied to the dentinal surface of all samples according to the manufacturer's instructions, air-dried and light-cured for 20 seconds. Resin composite (Filtek Z350, 3M) was placed incrementally using a Teflon mould (5 mm $\times$ 2.5 mm) and light-cured for 40 seconds. Specimens were stored in distilled water at 37 °C for 24 hours before testing. Ten samples per group were subjected to shear bond strength testing on a universal testing machine using a straight knife-edge chisel applied at the tooth-restoration

interface at a crosshead speed of 1.0 mm/min until failure; values were recorded in MPa. Five samples per group were prepared for SEM by longitudinal sectioning through the resin build-up and dentin into two halves to expose the resin-dentin interface. Specimens were placed in 4% NaOCl for 20 minutes, treated with 20% HCl for 30 seconds, rinsed with distilled water, dehydrated sequentially in ascending grades of ethanol (60%, 70%, 80%, 90% for 20 minutes each and 100% for 1 hour), dried, mounted on aluminium stubs, sputter-coated with aluminium and observed under a scanning electron microscope at magnifications of 2000×, 5000× and 10000×. Resin tag length was measured on the photomicrographs using the scale bar as reference. Qualitative grading of resin tag formation was performed using the four-step (0–3) scale: Score 0 – no resin tag formation; Score 1 - few and short resin tags; Score 2 -long resin tags visible; Score 3 - dense resin tags with numerous lateral branches. All measurements were analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test for pairwise comparisons; the Kruskal-Wallis and Fisher's exact tests were used for the resin tag grading scores. Statistical significance was set at $p < 0.05$.

Results:

Quantifiable shear bond strength values, resin tag grading scores and resin tag lengths were recorded for all specimens. Mean values, standard deviations and statistical comparisons. All experimental groups treated with dentin bio modifiers demonstrated significantly higher shear bond strength than the untreated control group ($p < 0.001$). Group II (GSE) recorded the highest mean shear bond strength, followed by Group IV (GSE + CPP-ACP) and Group III (CPP-ACP) (Table 1). Multiple pairwise comparisons confirmed that the differences between all groups were statistically significant (Table 2). Visual grading of the resin-dentin interface revealed that the GSE-treated group exhibited the highest proportion of Score 3 (dense resin tags with numerous lateral branches), whereas Score 0 was observed only in the CPP-ACP group; the differences were statistically significant (Table III). Quantitative measurement of resin tag length showed that GSE produced significantly longer resin tags than all other groups (mean 70.20 μm), while the CPP-ACP group showed the shortest tags (Tables 4 and 5). The order of mean shear bond strength among the groups was: GSE > GSE + CPP-ACP > CPP-ACP > Control.

Table 1: Comparison of shear bond strength between the study groups

Group	N	Mean (MPa)	SD	Min	Max	p-value
Control	10	1.35	0.15	1.20	1.60	<0.001*
GSE	10	3.39	0.23	3.12	3.77	
CPP-ACP	10	2.54	0.25	2.20	2.96	
GSE + CPP-ACP	10	3.13	0.06	3.01	3.22	

*Statistically significant ($p < 0.05$); one-way ANOVA, $F = 234.95$

Table 2: Intergroup comparison of shear bond strength (Tukey HSD post hoc test)

Group (I)	Group (J)	Mean Difference (I–J)	Std. Error	p-value
Control	GSE	-2.04	0.08	<0.001*
Control	CPP-ACP	-1.19	0.08	<0.001*
Control	GSE + CPP-ACP	-1.78	0.08	<0.001*
GSE	CPP-ACP	0.85	0.08	<0.001*
GSE	GSE + CPP-ACP	0.26	0.08	0.02*

CPP-ACP	GSE + CPP-ACP	-0.59	0.08	<0.001*
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*Statistically significant ($p < 0.05$)

Table 3: Distribution of grading scores for resin tags across the four study groups

Score	Control (n=5)	GSE (n=5)	CPP-ACP (n=5)	GSE + CPP-ACP (n=5)	p-value
0	0 (0%)	0 (0%)	2 (40%)	0 (0%)	0.004*
1	3 (60%)	0 (0%)	3 (60%)	2 (40%)	
2	2 (40%)	1 (20%)	0 (0%)	3 (60%)	
3	0 (0%)	4 (80%)	0 (0%)	0 (0%)	

*Statistically significant ($p < 0.05$); Fisher's exact test

Table 4: Comparison of resin tag length (μm) between the study groups

Group	N	Mean (μm)	SD	Min	Max	p-value
Control	5	40.40	5.60	34	49	<0.001*
GSE	5	70.20	4.32	65	77	
CPP-ACP	5	29.00	2.74	26	33	
GSE + CPP-ACP	5	39.00	3.94	33	44	

*Statistically significant ($p < 0.05$); one-way ANOVA, $F = 86.55$

Table 5: Intergroup comparison of resin tag length (Tukey HSD post hoc test)

Group (I)	Group (J)	Mean Difference (I–J)	Std. Error	p-value
Control	GSE	-29.80	2.70	<0.001*
Control	CPP-ACP	11.40	2.70	0.003*
Control	GSE + CPP-ACP	1.40	2.70	0.95 (NS)
GSE	CPP-ACP	41.20	2.70	<0.001*
GSE	GSE + CPP-ACP	31.20	2.70	<0.001*
CPP-ACP	GSE + CPP-ACP	-10.00	2.70	0.009*

*Statistically significant ($p < 0.05$); NS: Not Significant

Discussion:

The present study showed that dentin biomodification with grape seed extract (GSE) and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) significantly improved dentinal shear bond strength and resin-dentin interface characteristics compared with the untreated control. Among all groups, GSE produced the highest shear bond strength, the longest resin tags and the greatest resin tag formation scores, whereas CPP-ACP showed the lowest bond strength among the experimental groups but remained superior to the control. These findings suggest that collagen biomodification before adhesive application enhances resin infiltration and interfacial stability. The superior performance of GSE agrees with the findings of Hardan *et al.* [9], who reported enhanced dentin bond strength following collagen cross-linking with grape seed extract. Similarly, Banu *et al.* [10] observed improved resin-dentin bond strength and hybrid layer integrity after GSE pre-treatment. The beneficial effects of GSE are attributed to its proanthocyanidin content, which forms inter- and intramolecular collagen cross-links through hydrogen bonding and hydrophobic interactions. This stabilizes the collagen matrix, inhibits matrix metalloproteinases, preserves the hybrid layer and promotes stronger micromechanical adhesion. The increased resin tag length observed in the present study further supports improved adhesive penetration into dentinal tubules following collagen stabilization. Although CPP-ACP exhibited lower bond strength than GSE, it still significantly outperformed the untreated control. This observation is consistent with the study by Firouzmandi *et al.* [11], who also reported moderate improvement in dentin bond strength following CPP-ACP application. The comparatively lower performance may result from mineral precipitation within

dentinal tubules, which can partially occlude tubules and limit adhesive infiltration despite promoting remineralization. Likewise, Nozari *et al.* [12] showed that CPP-ACP pre-treatment enhances dentin bonding but produces less pronounced improvements than collagen cross-linking agents because its primary action is mineral deposition rather than collagen stabilization. The longer resin tags observed in the GSE group indicate deeper penetration of adhesive resin into dentinal tubules and improved micromechanical interlocking, whereas the shorter resin tags in the CPP-ACP, combination and control groups may reflect partial tubule occlusion or incomplete adhesive infiltration. The use of an ethanol-based fifth-generation adhesive may have further enhanced the interaction between proanthocyanidins and dentinal collagen, contributing to superior bond strength [13]. A limitation of this study is its *in vitro* design without thermocycling or long-term ageing. Future investigations should evaluate the long-term durability, anti-proteolytic effects and clinical performance of these dentin biomodifiers under simulated oral conditions.

Conclusion:

GSE pre-treatment produced the highest dentinal shear bond strength, the highest grading scores for resin tag formation and the longest resin tags, followed by GSE combined with CPP-ACP and CPP-ACP alone; all three bio modifier treatments performed significantly better than the untreated control. The application of selective dentin bio modifiers, particularly GSE, may therefore represent a useful adjunctive strategy to enhance dentinal bond strength during adhesive restorative procedures. Further *in vitro* and *in vivo* studies evaluating long-term bond durability, hybrid

layer stability and clinical behaviour are recommended to validate these findings.

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References:

- [1] Perdigão J. *Jpn Dent Sci Rev.* 2020 **56**:190. [PMID: 34188727]
- [2] Van Meerbeek B *et al.* *J Adhes Dent.* 2020 **22**:7. [PMID: 32030373]
- [3] Sultan S *et al.* *J Funct Biomater.* 2024 **15**:41. [PMID: 38391894]
- [4] Amin F *et al.* *Coatings.* 2022 **12**:1094. [DOI: 10.3390/coatings12081094]
- [5] Mokeem LS *et al.* *Biomedicines.* 2023 **11**:1256. [PMID: 37238927]
- [6] Fan J *et al.* *Discover Nano.* 2025 **20**:40. [PMID: 39961978]
- [7] Silva AC *et al.* *Lasers Med Sci.* 2019 **34**:1493. [PMID: 30830558]
- [8] Costa R *et al.* *Int J Mol Sci.* 2025 **26**:3488. [PMID: 40331951]
- [9] Hardan L *et al.* *Cells.* 2022 **11**:2417. [PMID: 35954261]
- [10] Banu MA *et al.* *Med Pharm Rep.* 2022 **95**:300. [PMID: 36060507]
- [11] Firouzmandi M *et al.* *Eur J Dent.* 2020 **14**:288. [PMID: 32503066]
- [12] Nozari A *et al.* *Clin Exp Dent Res.* 2025 **11**:e70131. [PMID: 40260838]
- [13] Mithiborwala S *et al.* *Contemp Clin Dent.* 2012 **3**:158. [PMID: 22919214]

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