

Effect of nano-hydroxyapatite incorporation on the immediate and long-term bond stability of a one-step self-etch adhesive

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Abstract

Objective: This study evaluated the effect of incorporating nano-hydroxyapatite (nHAp) at various concentrations on the immediate and long-term bond strength of a one-step self-etch adhesive to dentin.

Methods: A total of 100 sound maxillary central incisors were collected. The dentin was exposed on the labial surfaces, and the teeth were randomly assigned into two groups (n=50) based on the evaluation time points: 24 hours and 6 months. Each group was further divided into five subgroups (n=10) based on the concentration of nHAp (0, 1, 2, 3, and 4 wt%) incorporated into Adper Single Bond Universal adhesive. Composite resin buildups were formed on dentin using cylindrical molds, and light-cured. Shear bond strength (SBS) was measured by a universal testing machine, and fracture modes were assessed under a stereomicroscope. Statistical analysis was performed using a two-way ANOVA ($P < 0.05$).

Results: At 24 hours, the highest bond strength was observed in 2% nHAp (41.68 ± 15.24 MPa), followed by 1% nHAp (36.75 ± 14.24 MPa), both significantly greater than the control group (0% nHAp: 17.03 ± 4.57 MPa; $P < 0.05$). After 6 months, 2% nHAp again demonstrated the highest bond strength (43.28 ± 6.87 MPa), followed by 1% nHAp (37.00 ± 6.90 MPa), both significantly higher than the control (15.28 ± 4.63) and 4% nHAp groups (14.58 ± 2.33 MPa; $P < 0.05$).

Conclusions: The incorporation of nano-hydroxyapatite at concentrations up to 2 wt% into a one-step self-etch adhesive significantly enhanced both immediate and long-term bond strength to dentin.

Keywords: Adper single bond, Bond strength, Composite resins, Dental cement, Dentin bonding agents, Nano-hydroxyapatite

Introduction

Modern adhesive systems are valued for their effectiveness in minimizing microleakage, which is essential for preventing secondary caries and increasing the longevity of restorations. Despite continual advancements in adhesive formulations and restorative materials, long-term clinical studies report failure rates of approximately 15–20% for tooth-colored restorations over 12 years (1). Therefore, improving the performance and durability of bonding agents remains a critical area of focus (2).

Bonding agents function by establishing a strong and stable interface between the tooth structure and composite resin, enabling the restoration to withstand mechanical forces and functional stresses. The

effectiveness of an adhesive system largely depends on its ability to establish micromechanical interlocking and/or chemical bonding with both enamel and dentin (3).

Over the years, adhesive systems have evolved significantly to enhance bond strength and simplify clinical protocols. Currently, two main strategies are employed to achieve adhesion: the etch-and-rinse (ER) technique and the self-etch (SE) approach (4). The self-etch approach provides considerable clinical benefits by eliminating the need for a separate acid-etching step, thereby simplifying the procedure and reducing both technique sensitivity and postoperative discomfort, particularly in dentin.

Self-etch adhesive systems are typically complex aqueous formulations containing functional monomers, cross-linking agents, monofunctional co-monomers, solvents, and various additives (5). These components synergistically demineralize the smear layer and superficial dentin while simultaneously infiltrating the substrate to form a hybrid layer. One-step self-etch adhesives, although convenient, are inherently

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hydrophilic and tend to behave like semi-permeable membranes after polymerization. This allows water movement from the underlying hydrated dentin into the adhesive layer, resulting in the formation of interfacial voids or “water trees”. This process can ultimately compromise the resin-dentin interface through hydrolytic degradation over time (6).

Various strategies have been proposed to counteract the degradation of the adhesive interface and enhance the longevity of the restoration. One such strategy is the incorporation of inorganic fillers. The inclusion of nano-sized fillers in self-etch adhesives has shown promise in improving mechanical strength, reducing permeability, and enhancing hydrolytic stability, all of which contribute to long-term bond durability (7, 8).

Nano-hydroxyapatite (nHAp), has attracted attention as a biocompatible filler to reinforce adhesive systems, due to its chemical similarity to the mineral component of dentin. Incorporating apatites into the adhesive improves its mechanical strength and promotes dentin remineralization, with these effects becoming more pronounced at the nanoscale (9,10). Moreover, studies have demonstrated that even small additions of hydroxyapatite powder can significantly improve the degree of conversion and polymerization rate in self-etch adhesives (11–13). Other studies reported enhanced bond stability with the incorporation of nanostructured hydroxyapatite, highlighting its potential as a functional additive in dental bonding systems (14,15).

Despite its benefits, the evidence regarding the influence of various concentrations of nHAp on the long-term bond stability of one-step self-etch adhesives remains limited and inconclusive. Therefore, the present in vitro study aimed to investigate the effect of incorporating nano-hydroxyapatite (0, 1, 2, 3, and 4 wt%) into a one-step self-etch adhesive on the immediate, and long-term bond strength of human dentin.

Materials and methods

Sample preparation

The sample size was calculated using G*Power software (Heinrich Heine University Düsseldorf, Düsseldorf, Germany) based on a previous study (16), with an alpha level of 0.05, a power of 0.80, and five experimental groups, a total of 50 specimens was required. Considering two evaluation time points (24 hours and 6 months), the sample size was doubled to 100.

One hundred non-carious human maxillary central incisors, extracted for periodontal or orthodontic reasons, were included in the study. Teeth with caries, coronal fractures, attrition, or developmental anomalies were excluded. The specimens were stored in 0.01% thymol solution until use.

Labial surfaces of the teeth were flattened using a diamond saw (Adler Instruments GmbH, Berlin, Germany) mounted on a lab micromotor handpiece (Saeyang Microtech Co., Daegu, South Korea) at low speed to expose dentin. Surface irregularities were removed using 600-grit silicon-carbide papers mounted on a mandrel. Roots were embedded vertically in acrylic resin blocks, leaving the crowns exposed up to the cemento-enamel junction.

Adhesive preparation

Adper Single Bond Universal adhesive (3M ESPE, St. Paul, MN, USA) was used as the base. Nano-hydroxyapatite powder (nHAp, particle size <200 nm; Sigma-Aldrich, USA) was incorporated into the adhesive in concentrations of 0%, 1%, 2%, 3%, and 4% by weight. Powder weights were measured using a precision microbalance (Ad-508, India), and adhesive volumes with 1 mL glass syringes. The nHAp powder and adhesive were mixed in a sealed container to prevent solvent evaporation. The mixture was then homogenized using probe sonication (Sonoplus UW2200; Bandelin, Berlin, Germany) for 1 minute, employing intermittent pulsing to minimize heat generation and solvent loss.

Study groups

Teeth were randomly assigned into two groups (n = 50 per group). Group 1 consisted of specimens that were evaluated after 24 hours of storage, and Group 2 included teeth that were evaluated after 6 months of storage.

Each group was further divided into five subgroups (n=10) based on the concentration of nano-hydroxyapatite (nHAp) incorporated into the adhesive:

- Group A: Control (0 wt% nHAp).
- Group B: 1 wt% nHAp.
- Group C: 2 wt% nHAp.
- Group D: 3 wt% nHAp.
- Group E: 4 wt% nHAp

Bonding procedure

The exposed dentin surfaces were rinsed thoroughly with water to remove debris and gently air-dried to maintain surface moisture. The adhesive was applied to

the dentin surface using a micro-applicator (Dochem, Shanghai, China), rubbed for 20 seconds, air-thinned for 5 seconds, and light-cured for 10 seconds with an LED curing unit (Bluephase; Ivoclar Vivadent, Schaan, Liechtenstein). A polyethylene tube mold (2 mm in diameter and 3 mm in height) was held on dentin. Composite resin (Filtek Z350; 3M ESPE) was placed in two increments into the tube and each layer was light-cured for 20 seconds.

Specimens in group 1 were stored in distilled water at 37°C for 24 hours, and those in group 2 for 6 months, with weekly water replacement.

Shear bond strength testing

Bond strength testing was performed by a universal testing machine (AGS-10kN; Shimadzu, Kyoto, Japan) equipped with a 2 mm-wide chisel attached to the upper jig. Specimens were mounted in the lower jig with the chisel aligned parallel to the composite–dentin interface. The load was applied at a crosshead speed of 1 mm/min until bond failure. Data were recorded in Newtons and then converted to MPa.

The mode of failure was determined using a stereomicroscope at 40× magnification and classified into three categories: adhesive (failure at the resin–dentin interface), cohesive (failure within the dentin or the adhesive material, and mixed (a combination of adhesive and cohesive failures).

Statistical analysis

Statistical analysis was performed using SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test confirmed the normal distribution of the data ($P > 0.05$). Bond strength values were compared

using a two-way analysis of variance (ANOVA), and a P -value < 0.05 was considered statistically significant.

Results

Table 1 presents the mean shear bond strength values in the study groups at 24-hour and 6-month storage intervals.

Two-way ANOVA revealed a significant interaction between the two variables ($P = 0.005$). Therefore, one-way ANOVA and independent samples t -tests were used for data analysis.

ANOVA revealed statistically significant differences among the study groups in the immediate SBS testing, ($P < 0.001$). Tukey's post hoc test showed that groups 1B and 1C demonstrated significantly higher bond strength compared to the control group ($P = 0.01$ and $P < 0.001$, respectively). However, there was no significant difference between groups 1B and 1C ($P = 0.833$), nor among groups 1D, 1E, and 1A ($P > 0.05$).

In the long-term SBS testing, one ANOVA again indicated significant differences among the groups ($P < 0.001$). Post hoc analysis revealed that groups 2C and 2B had significantly higher bond strength values than groups 2A and 2E ($P < 0.001$). Group 2C also showed a significantly higher SBS value than 2D ($P = 0.005$). No significant difference was found in other comparisons ($P > 0.05$; Table 1).

A comparison between storage times revealed no significant difference in bond strength for most groups. However, group 1E exhibited significantly higher bond strength in the immediate testing (20.09 ± 5.51 MPa) compared to its 6-month counterpart (14.85 ± 2.33 MPa) ($P = 0.013$; Table 1).

Table 1. Mean and standard deviation (SD) of shear bond strength (MPa) in different groups after 24 hours and 6 months of storage

Subgroup	Definition	Group 1 (24-hours)	Group 2 (6-months)	P-value
		Mean $\pm$ SD	Mean $\pm$ SD	
A	0 wt % nHAp (Control)	17.03 ± 4.57^a	15.28 ± 4.63^a	0.405
B	1 wt % nHAp	36.75 ± 14.24^b	37.00 ± 6.90^{bc}	0.962
C	2 wt % nHAp	41.68 ± 15.24^b	43.28 ± 6.87^c	0.765
D	3 wt % nHAp	25.79 ± 8.30^{ab}	25.14 ± 8.23^b	0.864
E	4 wt % nHAp	20.09 ± 5.51^{ab}	14.85 ± 2.33^a	0.013
P-value		< 0.001	< 0.001	

Different lowercase superscript letters indicate significant differences between groups at $P < 0.05$.

The failure modes, as observed under stereomicroscopy, are summarized in Table 2. In the 24-hour storage, cohesive failure was most predominantly observed in groups 1B (80%), 1C (80%), 1D (70%), and 1E (60%), whereas group 1A exhibited a higher proportion of mixed failure (40%). After 6 months of storage, cohesive failure was most prevalent in groups 2B (50%) and 2C (50%). Mixed failure dominated in groups 2E (70%), 2D (60%), and 2A (40%).

Discussion

The present study evaluated the effect of various concentrations of nHAp on the long-term bond stability of a one-step self-etch adhesive. The results showed that the incorporation of nano-hydroxyapatite (nHAp) into the self-etch adhesive improved the shear bond strength (SBS) values compared to the control group (self-etch adhesive without nHAp). Among the tested concentrations, adhesives containing 1 wt% and 2 wt% nHAp demonstrated the highest SBS, after which bond strength declined with increasing filler content.

The nHAp powder used in this study had a grain size of less than 200 nm. Nanoparticles of hydroxyapatite exhibit high surface activity and an ultrafine structure that closely resembles the mineral composition of natural hard tissues (17). nHAp particles are biocompatible, release remineralizing ions such as calcium and phosphate, and demonstrate a strong affinity for tooth substrates (7). Their heterogeneous rod-like structure increases surface area, enhancing both mineral attachment and release. Furthermore, nHAp improves the resistance of the adhesive interface to degradation by reducing hydrophilicity, inhibiting enzymatic collagen breakdown, and minimizing stress-induced shrinkage (18).

The results of the present study demonstrated that the highest mean shear bond strength (SBS) was achieved in the group containing 2 wt% nano-hydroxyapatite (nHAp), followed by the 1 wt% nHAp group at both immediately (after 24 hours) and after 6 months of storage. The observed improvement in SBS with 2 wt% nHAp is consistent with the findings of Leitune et al. (15), who reported enhanced micro shear bond strength at this concentration due to optimal mechanical reinforcement and efficient resin infiltration. They also found that the degree of conversion (DC) remained within clinically acceptable limits, indicating adequate polymerization. Therefore, 2 wt% nHAp appears to provide an optimal balance between mechanical reinforcement and polymerization efficiency and may be recommended for clinical applications.

The increase in SBS upon nHAp incorporation may be partially attributed to an increased DC and polymerization rate. Previous studies employing ATR/FT-IR spectral analysis have shown that hydroxyapatite can accelerate polymer network formation by offering additional reactive sites for polymerization (13–15).

In the present study, further increases beyond 2 wt% in nHAp concentration led to a decline in SBS, which can be explained by several factors. A higher filler content may increase adhesive viscosity, thereby reducing its ability to effectively penetrate dentinal tubules. Moreover, the agglomeration of nHAp particles at elevated concentrations can create local inhomogeneities that weaken the adhesive interface (11). Additionally, incorporating excessive amounts of nHAp into the adhesive can increase its pH, compromising the self-etching ability by reducing effective dentin demineralization (13). Furthermore, the presence of opaque nHAp particles can scatter visible light, leading to reduced light transmission through the

Table 2. Failure mode frequency (percentage) in different groups after 24 hours and 6 months of storage

Subgroup	Definition	Group 1 (24-hours)			Group 2 (6-months)		
		Cohesive	Adhesive	Mixed	Cohesive	Adhesive	Mixed
A	0 wt % nHAp (Control)	3 (30%)	3 (30%)	4 (40%)	3 (30%)	3 (30%)	4 (40%)
B	1 wt % nHAp	8 (80%)	1 (10%)	1 (10%)	5 (50%)	0 (0%)	5 (50%)
C	2 wt % nHAp	8 (80%)	1 (10%)	1 (30%)	5 (50%)	1 (10%)	4 (40%)
D	3 wt % nHAp	7 (70%)	1 (10%)	2 (20)	1 (10%)	3 (30%)	6 (60%)
E	4 wt % nHAp	6 (60%)	0 (0%)	4 (40%)	0 (0%)	3 (30%)	7 (70%)

adhesive. This limits the depth of cure and impairs polymerization, especially at higher nHAp concentrations (12).

The outcomes of delayed bond strength testing revealed that the SBS values were generally maintained over the 6-month storage period. Only the 4 wt% nHAp group exhibited a noticeable decrease in SBS over time, which may be attributed to its elevated viscosity hindering effective resin infiltration into dentinal tubules. This likely compromised hybrid layer formation and polymerization depth, leading to reduced bond durability under prolonged storage.

Self-etch adhesives are unable to fully replace water within the collagen matrix with resin monomers, resulting in weak points within the hybrid layer. Biomimetic mineralization addresses this issue by depositing nanoscale apatite crystallites into these water-rich, resin-deficient regions. The nHAp acts as a mineral scaffold and regulates apatite formation (19,20). This targeted mineral deposition preserves the collagen network, enhances hybrid layer stability, and promotes long-term bond durability (21,22). Establishing a mineral-rich environment at the adhesive interface helps to exclude proteolytic enzymes and reduces the risk of long-term degradation (23).

The adhesive used in this study, Adper Single Bond Universal (3M ESPE), contains MDP (methacryloyloxydecyl dihydrogen phosphate). MDP is a functional monomer with a hydrophilic phosphate group and a hydrophobic long-chain methacrylate. It can chelate with calcium in hydroxyapatite, forming a stable MDP-Ca salt that reinforces chemical adhesion, thereby improving bond strength and long-term durability (24, 25).

The findings of this study highlight the critical need to optimize the concentration of nHAp in self-etch adhesives to achieve a balance between bioactivity and adhesive performance. Incorporating up to 2 wt% nHAp was shown to significantly improve bond strength and hybrid layer integrity, likely due to enhanced mechanical reinforcement. However, exceeding this threshold compromises adhesive performance by increasing viscosity, which potentially impairs monomer infiltration and polymerization efficiency. The increased nHAp concentration also may lead to nanophase separation, a reduced depth of cure, and increased susceptibility to hydrolytic degradation (16).

Generally, adhesive failure is defined as separation occurring at the resin–dentin interface, indicating inadequate bonding between the adhesive and the tooth surface. Cohesive failure refers to fractures

occurring entirely within the dentin or the adhesive material, suggesting that the bond strength exceeded the internal strength of one of the substrates. Mixed failure involves a combination of adhesive and cohesive characteristics, with fracture propagating through both the adhesive interface and one of the bonded substrates. At the immediate bond testing, predominantly cohesive failures were observed across all of the 1 wt% to 4 wt% nHAp groups, indicating strong initial bonding and effective resin infiltration within the hybrid layer.

After 6 months of storage, notable differences in failure patterns were observed. Cohesive failures remained dominant in the 1 wt% and 2 wt% groups, potentially reflecting sustained bond strength and hybrid layer stability over time. In contrast, the 3 wt% and 4 wt% groups exhibited a shift toward more mixed and adhesive failures, implying a decline in long-term bond durability. This change may be attributed to increased viscosity and reduced monomer infiltration at higher nHAp concentrations, which can impede polymerization and promote nanophase separation.

Despite efforts to simulate clinical conditions, this *in vitro* study has inherent limitations. Factors such as static storage and the absence of thermocycling do not fully replicate the dynamic and complex nature of the oral environment, thereby limiting the direct clinical applicability of the findings. Future studies should explore the effects of nano-hydroxyapatite on adhesive pH, matrix metalloproteinase (MMP) inhibition, polymerization kinetics, and the long-term performance of adhesives under thermomechanical stress.

Conclusions

Incorporating 1 wt% and 2 wt% nano-hydroxyapatite (nHAp) into a one-step self-etch adhesive significantly enhanced both immediate and long-term shear bond strength, potentially leading to improved adhesion, reduced risk of restoration failure, and long-term interface stability. However, higher concentrations of nHAp (3 wt% and 4 wt%) adversely affected adhesive performance, likely due to increased viscosity and reduced resin penetration.

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None to report.

Conflict of Interest

The authors declare that they have no competing interests.

Authors contributions

M.V.K. conceptualized, designed, and supervised the study and, reviewed the manuscript; B.P.V. conceptualized and designed the study, and performed data acquisition; K.S.R. assisted with study design and conducted the literature review; J.B.M. interpreted the results, assisted with data analysis, drafted the manuscript, and contributed to manuscript revision; G.S.S. interpreted the results and contributed to drafting the manuscript; V.P. assisted with data analysis and manuscript revision.

Ethical approval

There are no animal or human subjects involved in this article.

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