J Dent Mater Tech 2025; 15 (2): 81-87
DOI: 10.22038/jdmt.2026.94707.1976

J Dent Mater Tech

Original Article Open Access

Bond strength of an epoxy resin-based sealer modified with nano-hydroxyapatite and beta tricalcium phosphate to root dentin

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Abstract

Objective: This study evaluated the push-out bond strength of an epoxy resin-based sealer (AH Plus®) modified with nano-hydroxyapatite (n-HA) and β-tricalcium phosphate (β-TCP), compared with an unmodified control group.

Methods: In this in vitro study, β-tricalcium phosphate and nano-hydroxyapatite were synthesized by wet chemical precipitation, sintered, and then combined to obtain biphasic calcium phosphate. The prepared biphasic calcium phosphate was subsequently incorporated into AH Plus sealer at a concentration of 2.5 wt%. Forty-six maxillary anterior teeth were instrumented and randomly assigned to two groups: unmodified AH Plus sealer (control group) and AH Plus sealer modified with 2.5 wt% biphasic calcium phosphate (experimental group). Obturation was performed using the single-cone technique. After storage at 37°C and 100% humidity for 48 hours, 1-mm-thick root slices were prepared from the coronal, middle, and apical thirds and subjected to push-out bond strength testing using a universal testing machine. Failure modes were evaluated under a stereomicroscope.

Results: The experimental group showed significantly higher push-out bond strength than the control group (138.74 ± 19.44 N vs. 120.65 ± 19.52 N; P=0.003). Cohesive failure was the most frequent failure mode in the experimental group (39.1%), whereas adhesive failure was the most frequent in the control group (43.5%). However, the difference in failure modes was not statistically significant between the groups (P>0.05).

Conclusions: The incorporation of β-TCP and n-HA as biphasic calcium phosphate into AH Plus sealer was associated with significantly higher push-out bond strength compared with the unmodified control, indicating improved resistance to dislodgement from radicular dentin.

Keywords: Canals sealer, Dental cements, Hydroxyapatites, Materials testing, Root canal obturation, Tricalcium phosphate

Introduction

The main goal of a contemporary endodontic sealer is to establish a biologically stable and mechanically durable interface with root canal dentin. The performance of a sealer depends not only on its sealing ability but also on its capacity to bond effectively to dentin and withstand functional stresses (1). Since their introduction by Schroeder, epoxy resin-based sealers have been regarded as the gold standard in endodontics (2). These sealers are known for their excellent physical properties, biocompatibility, radiopacity, strong adhesion, and long-term dimensional stability (3, 4). Recent studies have further explored these materials and found that the incorporation of bioactive components or inorganic nanofillers can improve the performance of resin-based sealers.

The combination of β-tricalcium phosphate (β-TCP) and nano-hydroxyapatite (n-HA) forms a biphasic calcium phosphate (BCP) material with promising bioactive and mineralizing potential for dental applications (5). β-tricalcium phosphate (β-TCP) is a single-phase calcium phosphate ceramic composed of $Ca_3(PO_4)_2$. It is relatively resorbable and can release calcium and phosphate ions, thereby contributing to bioactivity and mineral deposition. Hydroxyapatite (HA) is chemically similar to the mineral phase of dental hard tissues and bone and is less soluble than β-TCP. When used in nanoscale form, nHA provides a high surface area, which may enhance interaction with biological fluids and favor apatite nucleation (6). The dual-phase structure of biphasic calcium phosphate combines the greater resorbability and ion-releasing ability of β-TCP with the higher stability and apatite-forming potential of HA, resulting in controlled degradation and sustained biological performance.

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Accepted: 29 May 2026. Submitted: 17 February 2026

Previous studies have shown that β-tricalcium phosphate may enhance the sealing ability of adhesive systems by releasing calcium and phosphate ions, which support apatite nucleation and interfacial mineral deposition, leading to improved micromechanical interlocking at the dentin surface (7, 8). Camargo et al (9) reported that adding β-TCP to an epoxy resin–based sealer improved its biocompatibility and bond strength to radicular dentin. Other studies indicated that the incorporation of nanomaterials such as n-HA into resin-based sealers may increase surface reactivity, contribute to antibacterial effects, and improve interaction with radicular dentin (10).

Biphasic calcium phosphate has also been investigated for restorative and regenerative dental applications (11, 12). However, evidence regarding the incorporation of biphasic calcium phosphate into AH Plus sealer remains limited. Therefore, the present in vitro study aimed to evaluate the push-out bond strength of biphasic calcium phosphate–modified AH Plus sealer compared with unmodified AH Plus sealer.

Materials and methods

Study design and ethical considerations

This in vitro study was conducted at the Department of Conservative Dentistry and Endodontics, Sathyabama Institute of Science and Technology, Chennai, India. The study protocol was approved by the Sathyabama Ethics Committee under the approval code of 473.

Sample size calculation

The sample size was calculated using G*Power software (version 3.1.9.4; Heinrich-Heine University Düsseldorf, Düsseldorf, Germany). Based on a one-tailed test, a significance level (α) of 0.05, a statistical power of 80%, and a Cohen's d effect size of 0.80, the minimum required sample size was estimated to be 42 specimens in total, with 21 specimens allocated to each group.

Preparation of β-Tricalcium Phosphate (β-TCP)

A solution was prepared by dissolving 25.765 g of ammonium hydrogen phosphate ($(NH_4)_2HPO_4$; Sigma-Aldrich, MA, USA) in 325 mL of distilled water. The ammonium hydrogen phosphate solution was gradually added to a solution containing 69.675 g of calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$; Sigma-Aldrich) dissolved in 500 mL of distilled water, according to a wet chemical precipitation method described previously (13). Then, 16.5 mL of ammonia was added, and the mixture was stirred continuously at 37°C for 2 hours. The resulting precipitate was filtered, thoroughly washed with distilled water, dried at 60°C for 24 hours, ground into a fine powder, and calcined at 850°C for 12 hours to obtain β-TCP powder.

Preparation of nano-hydroxyapatite (n-HA)

Based on a previously described method (14), a 0.5 M calcium hydroxide solution was prepared by dissolving 17.725 g of calcium hydroxide ($Ca(OH)_2$; Sigma-Aldrich) in distilled water and adjusting the final volume to 500 mL. Separately, a 0.3 M phosphoric acid solution was prepared by diluting 9.87 mL of phosphoric acid (H_3PO_4; Sigma-Aldrich) with distilled water to a final volume of 500 mL. The phosphoric acid solution was added dropwise to the calcium hydroxide solution under continuous stirring until the pH reached 12.5.

After 24 hours of continuous stirring, the precipitated material was collected by centrifugation. The precipitate was repeatedly washed with distilled water to remove unreacted precursors and soluble by-products, filtered, and dried. The dried material was then sintered in a muffle furnace for 1 hour to produce n-HA powder. The phase purity of the synthesized n-HA was confirmed by X-ray diffraction (XRD) using reference data from the Joint Committee on Powder Diffraction Standards (JCPDS). Scanning electron microscopy (SEM) was used to evaluate particle morphology, size, surface characteristics, and agglomeration.

Preparation of biphasic calcium phosphate

Biphasic calcium phosphate was prepared by combining nano-hydroxyapatite (nHAP) and β-tricalcium phosphate (β-TCP) in a 60:40 ratio. The two powders were weighed accurately and blended thoroughly to obtain a homogeneous composite with uniform particle distribution. The mixture was then used as the experimental BCP material for incorporation into the sealer.

Formulation of experimental sealer

Biphasic calcium phosphate was incorporated into the epoxy resin-based sealer (AH Plus®; Dentsply DeTrey GmbH, Konstanz, Germany) at a concentration of 2.5 wt%. This concentration was selected because it provided optimal physical and mechanical properties in pilot testing. The sealer was mixed according to the manufacturer's instructions using a 1:1 paste-to-paste ratio and was then used for sample obturation.

Sample selection and preparation

Forty-six freshly extracted single-rooted maxillary anterior teeth were collected. The included teeth were extracted for periodontal reasons and had a single root and canal with fully formed apices. Teeth with caries, cracks, root resorption, previous endodontic treatment, or root curvature greater than 5° according to Schneider's criteria (15) were excluded. All teeth were cleaned of soft tissue debris and stored in distilled water until use.

Access cavities were prepared using round diamond burs (JOTA AG, Rüthi, Switzerland), and the crowns were sectioned with a diamond disc (Abrasive Technology, Ohio, USA) to standardize the root length to 17 mm. Root canals were instrumented using ProTaper Gold rotary files (Dentsply Sirona, Ballaigues, Switzerland) up to size F4, and the working length was set at 1 mm short of the apex. Irrigation was performed with 2 mL of 5.25% sodium hypochlorite (NaOCl; Ultradent Products, South Jordan, Utah, USA) during instrumentation, followed by 3 mL of 17% EDTA (Ultradent Products) delivered at a flow rate of 1 mL/min. A final rinse with saline was performed to remove residual irrigants and minimize possible chemical interactions between the irrigating solutions and the sealer. All procedures were performed by a single experienced operator.

Grouping and obturation

The specimens were assigned unique codes and randomly allocated into two groups using a computer-generated random sequence (n = 23 per group). The groups were defined as follows:

- Control group: AH Plus®conventional sealer
- Experimental group: AH Plus sealer modified with 2.5 wt% biphasic calcium phosphate.

Root canal obturation was performed using the single-cone technique with matched 30/04 gutta-percha cones (Meta Biomed, Seoul, South Korea). The access cavities were restored with composite resin (Filtek™ Z250; 3M ESPE, St. Paul, MN, USA), and radiographs were taken to confirm the quality of obturation and the absence of voids. The specimens were stored at 37°C and 100% humidity for 48 hours to allow the sealer to set.

Sectioning and mechanical testing

Each specimen was sectioned horizontally to obtain 1-mm-thick slices from the coronal, middle, and apical thirds using a diamond disc (Abrasive Technology) under water cooling. Slice thickness was verified using a digital micrometer (Mitutoyo Corporation, Kawasaki, Kanagawa, Japan). Specimens with voids, irregular filling, or defects were excluded.

For the bond strength test, each section was positioned on a universal testing machine (Zwick/Roell ZOTEC, Einsingen, Germany), and the load was applied through a central plunger aligned with the root canal filling material until dislodgement occurred. The crosshead speed was set at 1 mm/min, and the maximum force (N) required to dislodge the filling was recorded (Figure 1).

Failure mode analysis

After testing, each specimen was examined under 40× magnification using a stereomicroscope (Leica Microsystems, Wetzlar, Hesse, Germany) by a blinded examiner. Failure mode was classified according to Seltzer's criteria (16) as adhesive (<25% sealer coverage on dentin), cohesive (≥75% sealer coverage on dentin), or mixed (25–75% sealer coverage).

Figure 2 represents a root section before and after bond strength testing.

Statistical analysis

Data were analysed using IBM SPSS Statistics software, version 27 (IBM Corp., Armonk, NY, USA). An independent samples t-test was used to compare the mean push-out bond strength between the unmodified AH Plus sealer and the biphasic calcium phosphate–

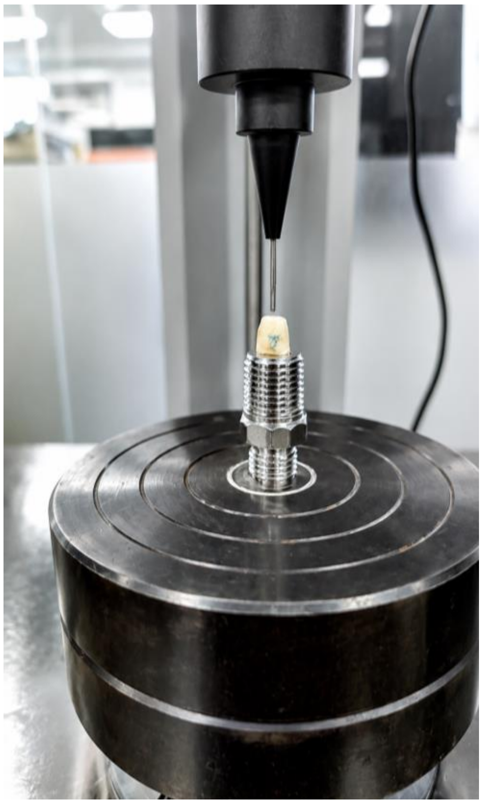

Figure 1. Experimental setup for the push-out bond strength testing at apical cross section using a universal testing machine

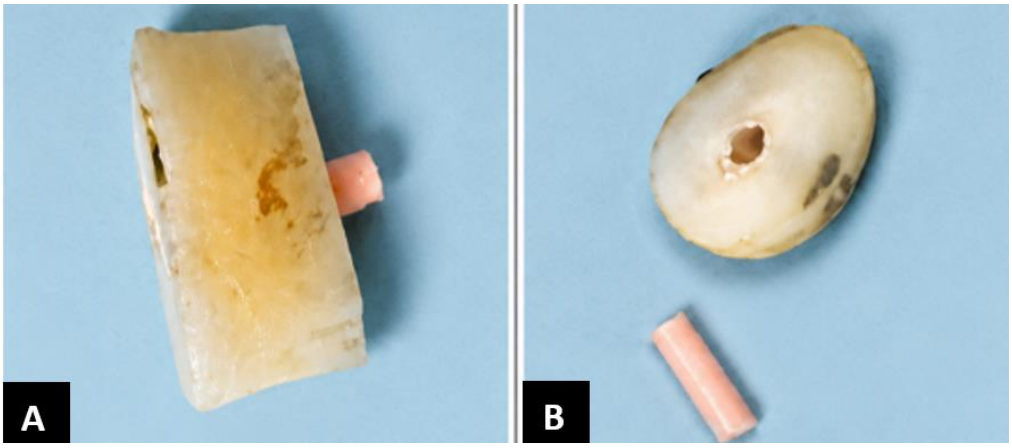

Figure 2. A representative mid-root section before (A) and after (B) push-out bond strength testing

modified AH Plus sealer. The distribution of failure modes between the groups was analysed using Pearson's chi-square test. A p-value < 0.05 was considered statistically significant.

Results

Table 1 presents the mean ± standard deviation (SD) values for push-out bond strength, along with the statistical comparison between the unmodified AH Plus sealer (control) group and the biphasic calcium phosphate–modified AH Plus sealer (experimental) group. The push-out load values for unmodified AH Plus and AH Plus modified with 2.5 wt% biphasic calcium phosphate were 120.65 ± 19.52 N and 138.74 ± 19.44 N, respectively. Statistical analysis using an independent samples t-test showed a significant difference between the two groups (P=0.003). The biphasic calcium phosphate–modified AH Plus sealer exhibited significantly higher bond strength than the conventional AH Plus sealer (Table 1).

In the AH Plus sealer modified with 2.5 wt% biphasic calcium phosphate group, cohesive failure was the most common pattern (39.1%). In the control group, adhesive failure was the most frequent pattern (43.5%). However, the difference in failure mode distribution between the groups was not statistically significant (P= 0.464; Table 2).

Discussion

The present in vitro study evaluated the effect of incorporating 2.5 wt% biphasic calcium phosphate, composed of β-tricalcium phosphate (β-TCP) and nano-hydroxyapatite (n-HA), into an epoxy resin–based root canal sealer (AH Plus) on push-out bond strength to radicular dentin. Bond strength was assessed using a push-out test on standardized root dentin slices prepared from extracted single-rooted maxillary anterior teeth. In addition to bond strength testing, fractured specimens were examined under a stereomicroscope to determine the mode of failure at the sealer–dentin interface. Standardization of the obturation protocol throughout the procedure helped minimize procedural variability.

The results of this study demonstrated that the biphasic calcium phosphate–modified AH Plus sealer exhibited significantly higher push-out bond strength than the control group (138.74 ± 19.44 N vs. 120.65 ± 19.52 N). The experimental group also showed a higher proportion of cohesive failure, whereas adhesive failure was more common in the control group. Although the difference in failure mode distribution was not

Table 1. The mean± standard deviation (SD) of push-out bond strength values (N) in the study groups

Group	Definition	Pull-put test Mean ± SD
Control group	unmodified AH Plus sealer	120.65 ± 19.52
Experimental group	biphasic calcium phosphate–modified AH Plus sealer	138.74 ± 19.44
P-value		0.003

Independent samples t test; P < 0.05 was considered statistically significant

Table 2. The frequency (N) and percentage (%) of failure modes in the study groups

Groups	Definition	Adhesive N (%)	Mixed N (%)	Cohesive N (%)
Control group	Unmodified AH Plus sealer	10 (43.5%)	6 (26.1%)	7 (30.4%)
Experimental group	biphasic calcium phosphate–modified AH Plus sealer	6 (26.1%)	8 (34.8%)	9 (39.1)
P value			0.464	

Chi square test; P < 0.05 was considered statistically significant.

statistically significant, the higher proportion of cohesive failure patterns may suggest improved interfacial bonding with radicular dentin.

The improved bond strength of the experimental group may be attributed to the bioactive and reinforcing effects of biphasic calcium phosphate. The release of calcium and phosphate ions from β-TCP and n-HA can promote apatite deposition at the dentin–sealer interface, which may contribute to interfacial sealing and micromechanical retention (17, 18).

From a clinical perspective, improved push-out bond strength may contribute to better sealer retention, fewer interfacial gaps, and improved long-term sealing ability. Cimpean et al. (19) demonstrated that sealers with higher push-out bond strength values showed significantly greater resistance to dislodgement. Strong adhesion between the sealer and radicular dentin is important for reducing microleakage and minimizing reinfection after endodontic treatment (20, 21).

The findings of the present study are consistent with previous reports on the incorporation of bioactive fillers and nanoparticles into dental materials (7-9, 22-24). Camargo et al. (9) reported improved adhesion to radicular dentin and enhanced biocompatibility after the incorporation of tricalcium phosphate into epoxy resin sealers. Huang et al. (24) developed a novel bioactive glass (BG)-based root canal sealer and reported favorable physicochemical properties, mineralization potential, sealing ability, and biocompatibility for endodontic treatment. In contrast, Waly and Salama (25) demonstrated improved bioactivity following the incorporation of bioactive fillers into epoxy resin–based sealers without significant changes in bond strength.

With respect to failure mode, the biphasic calcium phosphate–modified group demonstrated a higher proportion of cohesive failure, whereas adhesive failure was more frequent in the control group. Although the difference in failure mode distribution between the groups was not statistically significant, the higher proportion of cohesive failure in the biphasic calcium phosphate–modified group may suggest that the intrinsic strength of the modified sealer was exceeded before interfacial debonding occurred, indicating a possible improvement in sealer–dentin adhesion compared with the control group. Similar failure patterns have been reported for AH Plus and other bioceramic sealers, where cohesive failure was interpreted as evidence of stronger bonding to radicular dentin (26, 27).

Although increasing filler content can improve bond strength, excessive filler loading may lead to particle agglomeration, poor dispersion, and reduced resin–filler interaction, ultimately reducing material performance. Previous studies on adhesive and resin-based systems have shown that bond strength may increase at low to moderate filler levels but decline at higher concentrations (28-30). The present findings suggest that 2.5 wt% biphasic calcium phosphate may provide an optimal balance between reinforcement and adequate dispersion within the AH Plus matrix, as evidenced by the increased bond strength observed in the modified sealer group.

β-tricalcium phosphate and nano-hydroxyapatite are widely used bioactive calcium phosphate materials with established biocompatibility in dental applications, supporting their suitability as sealer additives (31). Both materials can be synthesized by wet chemical precipitation using readily available precursors, making laboratory preparation feasible (32, 33). However, before clinical use, further studies are needed to evaluate the effects of these additives on the physical properties of the final sealer formulation, including flow, working time, film thickness, and long-term biocompatibility.

The present study has certain limitations. As an in vitro investigation, the findings may not fully reflect the clinical environment. Only single-rooted maxillary anterior teeth were included, which limits extrapolation to teeth with more complex root canal anatomy. Other important sealer properties, such as flow, solubility, setting time, and long-term sealing performance, were not evaluated in this study. In addition, advanced interfacial analysis, such as scanning electron microscopy, was not performed. Future studies should investigate the long-term durability of biphasic calcium phosphate–modified epoxy resin sealers under thermal and mechanical stresses, evaluate their additional physicochemical and biological properties, and assess their performance in clinically relevant models and in vivo conditions.

Conclusions

The incorporation of biphasic calcium phosphate into AH Plus sealer was associated with significantly higher push-out bond strength compared with the unmodified control, indicating improved resistance to dislodgement from radicular dentin.

Acknowledgements

The authors would like to express their sincere gratitude to Pink Lab, Saveetha Dental College and Hospital, for their invaluable support and contribution to this work.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

M.S. developed the project, collected the data, helped with data analysis, and edited the manuscript. N.T. helped with data collection, analyzed the data, and wrote the manuscript. All authors read and approved the final manuscript.

Ethical considerations

The study protocol was approved by the Sathyabama Ethics Committee under the approval code 0f 473.

Funding

The research reported in this article was self-funded, and no external financial support was received for its conduct.

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