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Article

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Posted Date: November 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11485723/v1

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Additional Declarations: No competing interests reported.

Preventive and Therapeutic Effects of Aloe vera Gel Versus 5% Sodium Fluoride Varnish on Orthodontic White Spot Lesions: An In Vitro Study of Enamel Surface Microhardness, Laser Fluorescence, and Shear Bond Strength

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Abstract

Background: Orthodontic white spot lesions (WSLs) result from enamel demineralization around fixed appliances. Although fluoride varnish is widely used for prevention and remineralization, *Aloe vera* has emerged as a potential natural alternative.

Objective: To compare the preventive and therapeutic effects of *Aloe vera* gel and 5% sodium fluoride varnish on enamel demineralization and their effects on orthodontic bracket shear bond strength (SBS).

Materials and Methods: Seventy-five extracted human premolars were randomly assigned to five groups (n = 15): preventive control, preventive *Aloe vera*, preventive fluoride varnish, therapeutic *Aloe vera*, and therapeutic fluoride varnish. Following bracket bonding, specimens underwent standardized demineralization and treatment protocols. Enamel surface microhardness and DIAGNOdent laser fluorescence were assessed, while SBS was evaluated in the preventive groups. Data were analyzed using ANCOVA, between-group comparisons of therapeutic changes, and ANOVA with post hoc testing.

Results: Preventive microhardness loss was greatest in controls (116.38 VHN), followed by *Aloe vera* (57.88 VHN) and fluoride varnish (22.66 VHN), with significant differences among groups ($P < 0.001$). Therapeutic microhardness recovery was significantly greater with fluoride varnish than with *Aloe vera* (66.21 vs. 46.94 VHN; $P = 0.001$). Fluoride varnish also produced greater reductions in laser fluorescence ($P = 0.014$). However, SBS was significantly lower with fluoride varnish (6.20 ± 1.73 MPa) than with *Aloe vera* (8.25 ± 1.54 MPa) or controls (8.06 ± 1.53 MPa; $P < 0.01$).

Conclusions: Both interventions reduced enamel demineralization and improved surface microhardness. Fluoride varnish demonstrated superior enamel-related outcomes but lower bracket SBS. *Aloe vera* warrants further investigation as a potential adjunctive agent.

Keywords: *Aloe vera*; white spot lesions; fluoride varnish; enamel microhardness; orthodontic brackets; shear bond strength.

1. Introduction

White spot lesions (WSLs) are among the most common complications of fixed orthodontic treatment. They result from enamel demineralization associated with plaque accumulation around orthodontic brackets, which creates an acidic environment that favors mineral loss. These lesions may persist after treatment and compromise dental aesthetics. A systematic review reported a prevalence of approximately 55% among orthodontic patients, emphasizing the importance of effective preventive strategies [1].

Topical fluoride remains a widely used approach for preventing enamel demineralization and promoting remineralization. Sodium fluoride varnish provides prolonged fluoride availability at the enamel surface; however, its effectiveness may vary depending on application protocols and clinical conditions [2]. Consequently, interest has increased in alternative natural agents, including *Aloe vera*, which contains bioactive compounds with potential antimicrobial and enamel-protective properties. Preliminary investigations have explored its effects on artificially demineralized enamel, although evidence supporting its remineralizing potential remains limited [3].

Beyond enamel protection, preventive agents should preserve the mechanical integrity of orthodontic bracket adhesion. Certain enamel treatments may influence shear bond strength (SBS), potentially affecting bracket stability during orthodontic therapy [4]. Therefore, evaluating both enamel changes and bracket adhesion is important when assessing the suitability of preventive interventions.

Despite growing interest in *Aloe vera*, evidence directly comparing freshly prepared *Aloe vera* gel with sodium fluoride varnish under both preventive and therapeutic conditions remains limited. Furthermore, their combined effects on enamel surface microhardness, laser fluorescence, and orthodontic bracket SBS have not been adequately characterized. **Accordingly, this in vitro study aimed to compare the preventive and therapeutic effects of Aloe vera gel and 5% sodium fluoride varnish on experimentally induced WSLs and to evaluate their influence on orthodontic bracket SBS.** The null hypothesis was that no significant differences would exist between the interventions in enamel microhardness changes or bracket SBS.

2. Materials and Methods

2.1. Study Design

This in vitro experimental study was designed to compare the preventive and therapeutic effects of freshly prepared *Aloe vera* gel and 5% sodium fluoride varnish on artificially induced enamel white spot lesions (WSLs). The experimental design followed the principles of the Checklist for Reporting In Vitro Studies (CRIS) to ensure methodological transparency and reproducibility [5].

Seventy-five extracted human maxillary premolars were allocated equally into five experimental groups (n = 15). Three groups were assigned to the preventive experiment, whereas two groups underwent therapeutic intervention following artificial lesion induction. Enamel surface microhardness was designated as the

primary outcome, while laser fluorescence and orthodontic bracket shear bond strength (SBS) were evaluated as secondary outcomes.

The experimental allocation and assessment schedule are presented in Table 1, and the complete experimental workflow is illustrated in Figure 1.

2.2. Specimen Selection and Preparation

Seventy-five extracted human maxillary premolars with intact buccal enamel were selected. Teeth exhibiting caries, restorations, cracks, fluorosis, developmental defects, or previous orthodontic bonding were excluded.

Following extraction, the specimens were cleaned of residual soft tissue and calculus and stored in 0.1% thymol solution for no longer than one month. Before experimentation, the teeth were thoroughly rinsed with distilled water and examined under magnification to confirm enamel integrity.

Each specimen was assigned a unique identification code and embedded vertically in an acrylic resin block, leaving the crown exposed. The buccal enamel surfaces were cleaned using nonfluoridated pumice, rinsed, and dried before baseline assessment and bracket bonding.

2.3. Sample Size and Group Allocation

The experimental sample comprised 75 specimens distributed equally among five groups. The preventive experiment included an untreated control group (Group A), an *Aloe vera* group (Group B), and a fluoride varnish group (Group C). The therapeutic experiment comprised an *Aloe vera* group (Group D) and a fluoride varnish group (Group E).

A computer-generated randomization sequence was used to allocate specimens to the experimental groups. Allocation was performed independently of outcome assessment, and coded specimens were used throughout the experiment.

A total of 75 specimens were included in the study, with 15 specimens allocated to each of the five experimental groups. No formal a priori sample size calculation was performed. The sample size was determined pragmatically based on specimen availability and the experimental design.

2.4. Orthodontic Bracket Bonding

Standard stainless-steel premolar brackets with a 0.022-inch MBT prescription and a nominal base area of 11.1 mm^2 were used.

The central buccal enamel surface was etched with 37% phosphoric acid gel for 30 seconds, rinsed thoroughly, and air-dried. A light-cured orthodontic adhesive system was applied according to the manufacturer's instructions, and each bracket was positioned centrally on the buccal surface. Excess adhesive was removed before polymerization.

The adhesive was light-cured for 20 seconds using an LED curing unit with an irradiance of at least 1000 mW/cm^2. Following bonding, specimens were maintained in distilled water at 37°C for 24 hours.

Importantly, bracket bonding was completed before artificial lesion induction in all experimental groups. No additional enamel etching or bracket bonding was performed during the therapeutic phase, thereby avoiding interference with previously demineralized enamel.

2.5. Standardization of Enamel Assessment Sites

A standardized enamel assessment region was established adjacent to each orthodontic bracket. Laser fluorescence measurements were obtained at a predefined site approximately 1 mm gingival to the bracket margin, using a positioning guide to reproduce the probe location and angulation.

Surface microhardness measurements were performed within a separate standardized enamel window, avoiding areas covered by adhesive or affected by previous indentations. Repeated measurements were obtained at distinct, predefined locations within this window.

The assessment regions were maintained consistently throughout the experimental procedures to minimize variability associated with enamel location, surface characteristics, and measurement technique.

2.6. Preparation of Aloe vera Gel

Fresh mature leaves of *Aloe barbadensis* Miller were washed thoroughly, and the outer rind was removed. The inner translucent gel was separated from the yellow latex layer, homogenized, and filtered to obtain a uniform preparation.

The resulting gel was transferred into sterile, light-protected containers and stored at 4°C for a maximum of 24 hours. Fresh preparations were produced daily, and their pH was measured before application.

A standardized volume of 0.5 mL was applied to the designated enamel surface using a calibrated applicator. The gel remained in contact with the enamel for one minute before standardized rinsing with distilled water. Applications were performed twice daily throughout the relevant 14-day experimental period.

2.7. Sodium Fluoride Varnish Application

A commercially available 5% sodium fluoride varnish containing approximately 22,600 ppm fluoride was used as the reference intervention.

A standardized volume of 0.05 mL was applied to the designated enamel surface using a microbrush. The varnish remained in contact with the enamel for 30 minutes before removal according to the experimental protocol.

A single application was performed at the beginning of the preventive or therapeutic phase, depending on group allocation.

The interventions were evaluated according to their specified application regimens rather than equivalent exposure frequencies.

2.8. Artificial Demineralization and Remineralization Protocol

Artificial enamel demineralization was induced using a standardized pH-cycling model designed to reproduce alternating periods of mineral loss and recovery.

The demineralizing solution contained 2.2 mmol/L calcium chloride, 2.2 mmol/L sodium dihydrogen phosphate, and 50 mmol/L acetic acid, adjusted to pH 4.5.

The remineralizing solution contained 1.5 mmol/L calcium chloride, 0.9 mmol/L sodium dihydrogen phosphate, and 150 mmol/L potassium chloride, adjusted to pH 7.0.

Both solutions were prepared using analytical-grade reagents and deionized water. The pH was verified using a calibrated digital pH meter, and the solutions were maintained at 37°C and replaced daily.

Each 24-hour cycle consisted of six hours of demineralization followed by 18 hours of remineralization. Specimens were rinsed with distilled water between solution transfers.

The preventive experiment incorporated 14 consecutive days of pH cycling. For the therapeutic experiment, lesion induction preceded the 14-day treatment period.

2.9. Preventive Experimental Protocol

The preventive experiment included Groups A, B, and C, with 15 specimens per group.

Following baseline assessment and bracket bonding, Group A underwent the pH-cycling protocol without an active preventive agent. Group B received freshly prepared *Aloe vera* gel twice daily, whereas Group C received a single application of 5% sodium fluoride varnish before initiation of pH cycling.

All specimens were subjected to identical demineralization and remineralization conditions for 14 consecutive days.

At the end of the experimental period, enamel surface microhardness and laser fluorescence were reassessed. Subsequently, SBS testing was performed to evaluate the mechanical integrity of orthodontic bracket adhesion.

2.10. Therapeutic Experimental Protocol

The therapeutic experiment included Groups D and E (n = 15 per group). Following baseline assessment and orthodontic bracket bonding, all specimens underwent **14 consecutive days of artificial lesion induction** using the standardized pH-cycling protocol, consisting of 6 hours of demineralization and 18 hours of remineralization daily at 37°C. Surface microhardness and laser fluorescence were subsequently reassessed to establish post-demineralization values before treatment initiation.

Group D received freshly prepared *Aloe vera* gel (0.5 mL) twice daily for 14 days, whereas Group E received a single application of 5% sodium fluoride varnish (0.05 mL) at the beginning of the therapeutic period. Throughout treatment, specimens were maintained in the standardized remineralizing solution at 37°C, with daily solution replacement.

At the end of the 14-day therapeutic period, surface microhardness and laser fluorescence were reassessed using the same standardized measurement procedures. Therapeutic outcomes were determined from individual changes

between post-demineralization and post-treatment measurements. The experimental sequence and assessment time points are summarized in **Table 1 and Figure 1**.

2.11. Outcome Assessment

2.11.1. Enamel Surface Microhardness

Enamel surface microhardness was assessed using the Vickers indentation method. A load of 100 g was applied for 10 seconds, and three indentations were obtained at each assessment stage.

Indentations were positioned sufficiently far apart to prevent interference between measurements. The mean of the three readings was calculated for each specimen and expressed as Vickers hardness number (VHN).

Preventive specimens were assessed at baseline and after pH cycling. Therapeutic specimens were assessed at baseline, following lesion induction, and after treatment.

Microhardness loss in the preventive experiment and recovery during the therapeutic period constituted the principal enamel-related outcomes.

2.11.2. Laser Fluorescence

Laser fluorescence was measured using the DIAGNOdent Pen 2190 (KaVo, Biberach, Germany).

The device was calibrated according to the manufacturer's instructions before each measurement session. Specimens were cleaned, rinsed, and dried under standardized conditions before assessment.

The probe was positioned perpendicular to the predefined enamel assessment site, and three consecutive readings were recorded. Their mean was used for analysis.

Laser fluorescence was considered an indirect indicator of enamel changes rather than a direct measurement of mineral content.

2.11.3. Shear Bond Strength

SBS was evaluated in the three preventive groups using a universal testing machine.

Each specimen was secured in a mounting fixture, and a chisel-shaped loading blade was positioned as close as possible to the bracket–adhesive interface. A shear force was applied at a crosshead speed of 1 mm/min until bracket debonding occurred.

The maximum debonding force was recorded in newtons and divided by the bracket base area of 11.1 mm^2 to calculate SBS in megapascals:

SBS (MPa) = Debonding force (N) / Bracket base area (mm^2).

The test configuration was standardized across specimens, with consideration of established principles for laboratory assessment of dental adhesive bond strength [6].

2.12. Blinding and Measurement Reliability

Specimens were identified using numerical codes throughout the experimental procedures. The examiner responsible for enamel measurements was blinded to treatment allocation.

The same measurement procedures, assessment sites, and environmental conditions were maintained across experimental groups.

Three repeated measurements were obtained for microhardness and laser fluorescence at each assessment stage to reduce measurement variability.

The use of a standardized positioning guide further minimized differences in measurement location and probe angulation.

2.13. Statistical Analysis and Ethical Considerations

Statistical analysis was based on the individual tooth as the experimental unit. Continuous variables were summarized using means and standard deviations, and treatment effects were expressed using mean differences and 95% confidence intervals.

For the preventive experiment, final enamel surface microhardness was compared among Groups A, B, and C using analysis of covariance (ANCOVA), with baseline microhardness included as a covariate. Pairwise comparisons were adjusted using the Holm method.

Therapeutic effects were evaluated by calculating the change between post-demineralization and post-treatment measurements for each specimen. Differences

in microhardness recovery and laser fluorescence reduction between Groups D and E were assessed using independent-samples comparisons of individual treatment changes.

SBS values were compared among the three preventive groups using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test for pairwise comparisons.

Statistical significance was established at $P < 0.05$. All statistical comparisons were two-sided.

Ethical Considerations

The original study protocol was approved by the Ethics Committee of the Faculty of Dentistry, University of Aleppo (Approval No. 451821). Human premolars were obtained following clinically indicated orthodontic extractions, with informed consent obtained for their use in research. All methods were performed in accordance with the relevant institutional guidelines and applicable regulations governing research involving human biological specimens.

3. Results

3.1. Specimen Distribution and Baseline Characteristics

The dataset comprised 75 specimens distributed equally among five experimental groups (n = 15). Groups A, B, and C were included in the preventive experiment, whereas Groups D and E were included in the therapeutic experiment. All 75 specimen records contained the outcome measurements required for their respective analyses.

Baseline enamel surface microhardness was similar across the five groups, with mean values ranging from 326.34 to 333.59 VHN. Baseline laser fluorescence values ranged from 6.58 to 8.20 units. Descriptive characteristics and subsequent measurements are presented in Tables 2 and 4.

3.2. Preventive Effects on Enamel Surface Microhardness

Following the 14-day pH-cycling protocol, enamel surface microhardness decreased in all three preventive groups (Table 2). The untreated control group (A) showed a reduction from 329.99 ± 14.06 to 213.61 ± 17.20 VHN, corresponding to a mean loss of 116.38 VHN. The *Aloe vera* group (B) decreased from 326.34 ± 18.47 to 268.46 ± 21.69 VHN, with a mean loss of 57.88 VHN. The fluoride varnish group (C)

demonstrated the smallest reduction, from 332.91 ± 13.98 to 310.25 ± 14.20 VHN, corresponding to a mean loss of 22.66 VHN.

ANCOVA, adjusting final microhardness for baseline values, identified a statistically significant overall difference among the preventive groups ($F(2, 41) = 185.53$, $P < 0.001$). The model explained approximately 91.1% of the variance in final microhardness ($R^2 = 0.911$).

Holm-adjusted pairwise comparisons demonstrated higher final microhardness in Group B than in Group A (adjusted mean difference, 57.66 VHN; 95% CI: 47.65–67.66; $P < 0.001$). Group C also showed higher adjusted microhardness than Group A (94.39 VHN; 95% CI: 84.40–104.38; $P < 0.001$) and Group B (36.74 VHN; 95% CI: 26.62–46.85; $P < 0.001$).

The descriptive changes and adjusted between-group comparisons are presented in Table 2. These findings indicate differences in the extent of microhardness loss under the specified preventive regimens.

3.3. Preventive Effects on Laser Fluorescence

Laser fluorescence values increased following pH cycling in all preventive groups (Table 2).

Group A exhibited an increase from 8.20 ± 1.20 to 17.91 ± 1.39 units, corresponding to a mean increase of 9.71 units. In Group B, fluorescence increased from 7.57 ± 2.04 to 11.39 ± 2.57 units, with a mean increase of 3.82 units. Group C demonstrated the smallest increase, from 7.53 ± 1.61 to 9.07 ± 2.60 units, corresponding to a mean increase of 1.54 units.

Thus, the descriptive pattern of laser fluorescence changes was consistent with that observed for surface microhardness, with the greatest change in the untreated control group and the smallest change in the fluoride varnish group.

3.4. Shear Bond Strength of Orthodontic Brackets

Mean bracket shear bond strength (SBS) differed among the preventive groups (Table 3). Group A demonstrated an SBS of 8.06 ± 1.53 MPa, compared with 8.25 ± 1.54 MPa in Group B and 6.20 ± 1.73 MPa in Group C.

One-way ANOVA identified a statistically significant overall difference in SBS ($F(2, 42) = 7.47$, $P = 0.0017$).

Tukey's post hoc analysis showed that Group C had significantly lower SBS than Group A (mean difference, −1.86 MPa; 95% familywise CI: −3.29 to −0.44; P = 0.0076) and Group B (−2.05 MPa; 95% familywise CI: −3.47 to −0.62; P = 0.0032).

The difference between Groups A and B was not statistically significant (mean difference, 0.18 MPa; 95% familywise CI: −1.24 to 1.61; P = 0.9479).

Accordingly, the fluoride varnish group exhibited lower measured SBS than both the untreated control and *Aloe vera* groups, whereas the latter two groups did not show evidence of a difference in this analysis.

3.5. Therapeutic Effects on Enamel Surface Microhardness

In the therapeutic experiment, artificial lesion induction was followed by a reduction in surface microhardness in both groups (Table 4).

In Group D, baseline microhardness decreased from 333.59 ± 18.27 to 218.64 ± 24.43 VHN following lesion induction, representing a mean loss of 114.95 VHN. Subsequent *Aloe vera* treatment was associated with an increase to 265.59 ± 32.95 VHN, corresponding to a mean recovery of 46.94 VHN.

In Group E, microhardness decreased from 332.91 ± 18.28 to 219.68 ± 20.63 VHN following lesion induction, representing a mean loss of 113.23 VHN. Following fluoride varnish treatment, microhardness increased to 285.89 ± 25.35 VHN, corresponding to a mean recovery of 66.21 VHN.

The between-group comparison of individual post-lesion-to-final changes showed greater mean microhardness recovery in Group E than in Group D (mean difference, 19.27 VHN; 95% CI: 8.50–30.04; t(28) = 3.67; P = 0.0010).

Although microhardness increased following treatment in both groups, final mean values remained below their respective baseline values.

3.6. Therapeutic Effects on Laser Fluorescence

Laser fluorescence increased following artificial lesion induction and subsequently decreased during the therapeutic phase in both groups (Table 4).

In Group D, mean fluorescence increased from 6.58 ± 1.76 units at baseline to 15.49 ± 2.78 units following lesion induction. After *Aloe vera* treatment, fluorescence decreased to 10.65 ± 3.74 units, representing a mean reduction of 4.84 units from the post-lesion measurement.

In Group E, fluorescence increased from 7.25 ± 1.53 to 15.69 ± 3.71 units following lesion induction and subsequently decreased to 8.57 ± 4.83 units after fluoride varnish treatment. The mean reduction during the therapeutic phase was 7.12 units.

The between-group difference in fluorescence reduction was 2.28 units in favor of Group E (95% CI: 0.50–4.05; t(28) = 2.63; P = 0.0138).

Final fluorescence values remained numerically higher than baseline in both groups.

3.7. Summary of Statistical Findings

The primary preventive analysis identified differences in baseline-adjusted final enamel microhardness among the three preventive groups, with the highest adjusted values in the fluoride varnish group and the lowest in the untreated control group.

In the therapeutic experiment, fluoride varnish was associated with greater microhardness recovery and a larger reduction in laser fluorescence than *Aloe vera* under the investigated treatment protocols.

Conversely, SBS was lower in the fluoride varnish group than in either the untreated control or *Aloe vera* group. No statistically significant SBS difference was detected between the latter two groups.

These comparisons describe outcomes under the specific experimental conditions and application schedules investigated.

4. Discussion

The present in vitro model examined the preventive and therapeutic effects of freshly prepared *Aloe vera* gel and 5% sodium fluoride varnish on experimentally induced enamel white spot lesions (WSLs), while also evaluating orthodontic bracket shear bond strength (SBS). Three principal findings emerged. First, both interventions were associated with less enamel microhardness loss than the untreated control during preventive pH cycling, although fluoride varnish produced the more favorable outcome. Second, fluoride varnish was associated with greater microhardness recovery following established demineralization. Third, despite its more favorable enamel-related outcomes, the fluoride varnish group exhibited lower SBS than both the *Aloe vera* and untreated groups. These findings suggest that enamel protection and bracket adhesion should be considered separately when

evaluating preventive agents, rather than assuming that improvements in one outcome necessarily correspond to improvements in the other.

4.1. Preventive Effects on Enamel Demineralization

The preventive experiment demonstrated a clear separation among the treatment groups, with microhardness loss greatest in untreated specimens, intermediate following *Aloe vera* application, and lowest following fluoride varnish application (Table 2). This pattern is consistent with the established role of fluoride in reducing enamel dissolution and promoting mineral redeposition under cariogenic conditions. However, the magnitude of the observed differences should be interpreted within the constraints of the experimental model. In particular, the results reflect specific application regimens under controlled pH cycling rather than equivalent-dose comparisons of the two agents.

The relatively favorable outcome observed with fluoride varnish is broadly consistent with evidence supporting fluoride-based prevention of orthodontic WSLs. Nevertheless, clinical findings are less uniform than laboratory outcomes might suggest. The Cochrane review by Benson et al. [2] identified uncertainty regarding the effectiveness of individual fluoride delivery methods during fixed orthodontic treatment. A subsequent systematic review focusing on fluoride varnish reported a reduction in WSL occurrence with repeated applications, although the certainty of evidence was rated very low [7]. These findings support the biological plausibility of the present observations while emphasizing that favorable in vitro results cannot establish clinical effectiveness.

The intermediate response observed with *Aloe vera* is also noteworthy. Although the treated specimens experienced less microhardness loss than untreated controls, substantial demineralization remained evident. This distinction is important because reduced mineral loss does not necessarily indicate complete prevention or direct remineralization. The findings are compatible with a possible protective effect under the investigated conditions, but the experimental design does not establish the mechanism responsible.

Silva et al. [3] previously investigated an *Aloe vera*-containing dentifrice and a fluoride toothpaste using artificially demineralized bovine enamel. Both products increased surface microhardness during simulated brushing, without a statistically significant overall difference attributable to dentifrice type. In contrast, the present model showed a greater enamel-related response with fluoride varnish than with freshly prepared *Aloe vera* gel. This difference need not represent a contradiction, because the studies differed in enamel substrate, formulation, treatment delivery, and experimental exposure. Moreover, the earlier study incorporated mechanical

brushing and commercial dentifrice ingredients, making it difficult to isolate the contribution of *Aloe vera* itself. (SciELO)

4.2. Therapeutic Effects on Established Enamel Lesions

Following artificial lesion induction, both therapeutic groups demonstrated increased surface microhardness during the treatment period, with a significantly greater mean recovery in the fluoride varnish group (Table 4). The similarity of post-lesion mean microhardness values between groups suggests that the observed difference was not simply attributable to a large initial imbalance in lesion severity. Nevertheless, differences in individual lesion characteristics and the absence of direct mineral-content measurements limit more detailed interpretation.

Importantly, neither treatment restored mean microhardness to its baseline value. Therefore, the observed improvements should be described as **partial surface microhardness recovery**, rather than complete remineralization or restoration of intact enamel. Vickers microhardness reflects resistance to localized indentation and is sensitive to changes in surface characteristics, but it does not directly quantify the mineral distribution throughout the lesion body.

The therapeutic findings also require consideration of the remineralizing environment. Because specimens were maintained in a calcium- and phosphate-containing solution during treatment, some recovery could have occurred independently of the applied agents. The absence of an untreated therapeutic control prevents separation of treatment-specific effects from spontaneous recovery under these conditions. Consequently, the between-group comparison supports a difference between the two treatment regimens, but it cannot establish the absolute remineralizing contribution of either intervention.

4.3. Interpretation of Laser Fluorescence Findings

Laser fluorescence measurements generally followed the same direction as the microhardness outcomes. In the preventive experiment, fluorescence increased most markedly in untreated specimens, whereas the fluoride varnish group showed the smallest increase (Table 2). During therapeutic assessment, fluorescence decreased in both groups, with a greater reduction following fluoride varnish application (Table 4).

The agreement between these measurements provides complementary evidence of changes in enamel condition. However, the two techniques assess different properties and should not be regarded as interchangeable. Microhardness evaluates localized mechanical resistance, whereas DIAGNOdent records fluorescence signals

that may be influenced by lesion characteristics, surface deposits, moisture, and residual treatment materials. Accordingly, a reduction in fluorescence cannot independently confirm mineral deposition or lesion reversal.

This limitation is particularly relevant when comparing a plant-derived gel with a fluoride varnish, because the interventions may leave different surface residues. Although standardized cleaning and measurement procedures were specified, the available data do not establish whether treatment-related fluorescence interference was completely eliminated. The fluorescence findings should therefore be interpreted as supportive secondary observations rather than definitive evidence of remineralization.

4.4. Effects on Orthodontic Bracket Shear Bond Strength

An important finding was the significantly lower SBS observed in the fluoride varnish group compared with both the untreated control and *Aloe vera* groups (Table 3). In contrast, SBS did not differ significantly between *Aloe vera* and untreated specimens.

The lower SBS in the fluoride group warrants careful interpretation because previous investigations have not consistently demonstrated adverse effects of fluoride varnish on orthodontic bracket adhesion. Chauhan et al. [4], for example, reported no statistically significant SBS differences among conventional adhesive, fluoride-releasing adhesive, and fluoride-varnish groups. Similarly, an earlier investigation found that fluoride varnish did not significantly alter bracket bond strength when conventional or self-etching primer systems were used [8]. (PubMed)

Differences in experimental sequence may help explain these contrasting findings. In the present model, brackets were bonded before treatment and subsequently exposed to the preventive protocols, whereas some previous investigations assessed fluoride application in relation to the initial bonding procedure. Therefore, the present SBS outcome may reflect changes occurring during subsequent exposure and pH cycling rather than a direct effect of fluoride on initial adhesive polymerization or enamel conditioning.

Nevertheless, this explanation remains hypothetical. The study did not include microscopic examination of the adhesive interface or adhesive remnant index (ARI) assessment, and the available measurements cannot determine whether debonding occurred predominantly at the enamel–adhesive interface, bracket–adhesive interface, or within the adhesive material.

Furthermore, the absence of a statistically significant SBS difference between *Aloe vera* and untreated specimens should not be interpreted as evidence of equivalence. No equivalence margin or noninferiority analysis was specified. Similarly, although the fluoride group demonstrated lower mean SBS, the experiment cannot establish whether this difference would translate into a clinically meaningful increase in bracket failure.

4.5. Interpretation of the Combined Outcomes

Considering the enamel and bonding outcomes together reveals a potentially important distinction between the two interventions. Fluoride varnish was associated with the greatest preservation and recovery of surface microhardness, whereas *Aloe vera* demonstrated a smaller enamel-related response without a detectable difference in SBS relative to untreated controls.

However, these outcomes do not justify ranking the agents according to overall clinical suitability. The study was not designed to determine an optimal balance between enamel protection and bracket retention, and no clinically validated threshold was established for weighing these outcomes against one another.

Moreover, the agents were administered using different protocols: *Aloe vera* was applied repeatedly, whereas fluoride varnish was administered once. Consequently, the findings compare the complete experimental regimens rather than the intrinsic efficacy of equal exposures. Differences in contact time, retention, and formulation may all have contributed to the observed responses.

The results therefore support further investigation of *Aloe vera* as a potential adjunctive approach, but do not establish that it can replace fluoride varnish or provide equivalent enamel protection.

4.6. Study Strengths and Limitations

The experimental design incorporated several features intended to improve methodological consistency, including standardized specimen selection, bracket placement before lesion induction, predefined enamel assessment regions, and repeated measurements at distinct experimental stages. The inclusion of both preventive and therapeutic protocols also allowed the interventions to be examined under different enamel conditions.

Nevertheless, several limitations materially affect interpretation. First, the use of an artificial pH-cycling model cannot reproduce the full complexity of the oral environment, including biofilm metabolism, salivary proteins, dietary variation,

mechanical wear, and individual differences in oral hygiene. Second, the absence of a therapeutic control group prevents estimation of treatment effects beyond those attributable to the remineralizing medium alone. Third, the lack of chemical characterization of the freshly prepared *Aloe vera* gel limits reproducibility and prevents identification of any constituent associated with the observed response.

Additional limitations include the unequal intervention schedules, relatively small group sizes, absence of independent measurement-reliability estimates, and lack of ARI assessment. Surface microhardness and laser fluorescence also provide indirect information about enamel mineral changes; neither establishes the depth or structural distribution of remineralization.

4.7. Implications for Future Research

Further laboratory studies should incorporate an untreated therapeutic control, standardized exposure schedules, and chemical characterization of *Aloe vera* preparations. Additional methods, such as transverse microradiography or cross-sectional microhardness assessment, would help distinguish superficial changes from mineral recovery within the lesion body.

For orthodontic adhesion, future investigations should evaluate failure patterns alongside SBS and assess whether observed differences persist across adhesive systems and repeated exposure conditions. Ultimately, well-designed clinical trials would be required to determine whether laboratory differences translate into meaningful changes in WSL development, lesion appearance, or bracket failure during orthodontic treatment.

5. Conclusions

Within the limitations of this in vitro study, both *Aloe vera* gel and 5% sodium fluoride varnish were associated with reduced enamel microhardness loss under preventive conditions and partial microhardness recovery following artificial demineralization. Fluoride varnish demonstrated more favorable enamel-related outcomes in both experimental settings, whereas *Aloe vera* showed a smaller but measurable response.

However, fluoride varnish was associated with significantly lower orthodontic bracket shear bond strength, while no statistically significant difference was detected between *Aloe vera* and untreated controls. These findings highlight the importance of evaluating enamel protection alongside bracket adhesion.

Further controlled laboratory investigations, followed by clinical studies, are required to establish the effectiveness, reproducibility, and clinical relevance of *Aloe vera* as a potential adjunct to conventional fluoride-based strategies.

Funding

No funding was received for the conduct of this study.

Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Table Legends

Table 1. Experimental group allocation, treatment protocols, and outcome assessment schedule.

Distribution of 75 extracted human maxillary premolars into five experimental groups (n = 15 per group). Groups A–C underwent preventive protocols, whereas Groups D and E underwent artificial lesion induction followed by therapeutic interventions. The table summarizes treatment allocation, application frequency, experimental conditions, and assessment time points.

Abbreviations: NaF, sodium fluoride; SBS, shear bond strength.

Table 2. Preventive effects of Aloe vera gel and 5% sodium fluoride varnish on enamel surface microhardness and laser fluorescence.

Baseline and final measurements are expressed as mean ± standard deviation (SD). Changes represent the difference between final and baseline measurements. Between-group comparisons of final microhardness were performed using analysis of covariance (ANCOVA), adjusting for baseline values, with Holm-adjusted pairwise comparisons. Confidence intervals are reported at the 95% level.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; NaF, sodium fluoride; SD, standard deviation; VHN, Vickers hardness number.

Table 3. Shear bond strength of orthodontic brackets following preventive interventions.

Shear bond strength values are expressed as mean ± SD in megapascals. Overall differences were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Pairwise mean differences are accompanied by 95% familywise confidence intervals and adjusted *P* values.

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; MPa, megapascal; NaF, sodium fluoride; SBS, shear bond strength; SD, standard deviation.

Table 4. Therapeutic effects of Aloe vera gel and 5% sodium fluoride varnish following artificial lesion induction.

Surface microhardness and laser fluorescence were assessed at baseline, after artificial lesion induction, and following the therapeutic period. Values are expressed as mean ± SD. Treatment-related changes were calculated from post-lesion to final measurements. Between-group differences in these changes are presented with 95% confidence intervals and corresponding *P* values.

Abbreviations: CI, confidence interval; NaF, sodium fluoride; SD, standard deviation; VHN, Vickers hardness number.



Figure Legends

Figure 1. Experimental design and workflow.

Flow diagram illustrating specimen selection, random allocation of 75 extracted human premolars into five groups (n = 15), orthodontic bracket bonding, preventive and therapeutic protocols, and outcome assessments. Preventive groups underwent 14 days of pH cycling, whereas therapeutic groups underwent artificial lesion induction followed by 14 days of treatment. Surface microhardness and laser fluorescence were evaluated at the designated time points, while shear bond strength was assessed in the preventive groups.

Abbreviations: NaF, sodium fluoride; SBS, shear bond strength; WSL, white spot lesion.

Figure 2. Preventive changes in enamel surface microhardness and laser fluorescence.

Comparison of baseline and final enamel surface microhardness (A) and laser fluorescence (B) among untreated controls, *Aloe vera*-treated specimens, and 5% sodium fluoride varnish-treated specimens following 14 days of pH cycling. Data are presented as mean ± SD. Statistical comparisons of final microhardness were performed using ANCOVA adjusted for baseline values, followed by Holm-adjusted pairwise comparisons.

Abbreviations: ANCOVA, analysis of covariance; NaF, sodium fluoride; SD, standard deviation; VHN, Vickers hardness number.

Figure 3. Therapeutic changes in enamel surface microhardness and laser fluorescence.

Changes in enamel surface microhardness (A) and laser fluorescence (B) at baseline, after artificial lesion induction, and following treatment with *Aloe vera* gel or 5% sodium fluoride varnish. Data are presented as mean ± SD. Between-group comparisons were based on individual changes from post-lesion to post-treatment measurements.

Abbreviations: NaF, sodium fluoride; SD, standard deviation; VHN, Vickers hardness number.

Figure 4. Shear bond strength following preventive interventions.

Comparison of orthodontic bracket shear bond strength among untreated controls, *Aloe vera*-treated specimens, and 5% sodium fluoride varnish-treated specimens. Data are presented as mean ± SD. Statistical differences were evaluated using one-way ANOVA followed by Tukey's post hoc test.

Abbreviations: ANOVA, analysis of variance; MPa, megapascal; NaF, sodium fluoride; SBS, shear bond strength; SD, standard deviation.

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List of Abbreviations

Abbreviation	Definition
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
CI	Confidence interval
CRIS	Checklist for Reporting In Vitro Studies
LED	Light-emitting diode
MBT	McLaughlin–Bennett–Trevisi
MPa	Megapascal
NaF	Sodium fluoride
SBS	Shear bond strength
SD	Standard deviation
VHN	Vickers hardness number
WSL	White spot lesion

WSLs	White spot lesions

Table 1. Experimental group allocation, intervention protocols, and outcome assessment schedule

Group	Experimental condition	n	Intervention	Experimental protocol	Assessment time points	Outcomes
A	Prevention—control	15	No active treatment	14-day pH cycling	Baseline (T_0); day 14 (T_1)	SMH, LF, SBS
B	Prevention—*Aloe vera*	15	Fresh *Aloe vera* gel (0.5 mL, twice daily for 14 days)	14-day pH cycling	Baseline (T_0); day 14 (T_1)	SMH, LF, SBS
C	Prevention—fluoride	15	5% NaF varnish (0.05 mL, single application)	14-day pH cycling	Baseline (T_0); day 14 (T_1)	SMH, LF, SBS
D	Therapy—*Aloe vera*	15	Fresh *Aloe vera* gel (0.5 mL, twice daily for 14 days)	14-day lesion induction followed by 14-day remineralization	Baseline (T_0); post-lesion (T_1); post-treatment (T_2)	SMH, LF
E	Therapy—fluoride	15	5% NaF varnish (0.05 mL, single application)	14-day lesion induction followed by 14-day remineralization	Baseline (T_0); post-lesion (T_1); post-treatment (T_2)	SMH, LF

Table legend: Seventy-five extracted human maxillary premolars were allocated equally into five experimental groups (n = 15). Orthodontic brackets were bonded before experimental demineralization in all groups. Preventive groups (A–C) underwent 14 consecutive days of pH cycling, consisting of 6 hours of demineralization and 18 hours of remineralization daily. Therapeutic groups (D and E) underwent **14 days of identical pH cycling to induce artificial white spot lesions**, followed by 14 days of treatment under remineralizing conditions at 37°C.

Aloe vera gel was applied twice daily, whereas fluoride varnish was applied once at the beginning of the respective intervention period. Surface microhardness and laser fluorescence were assessed at the specified time points. Shear bond strength was evaluated only in the preventive groups after completion of pH cycling.

Abbreviations: LF, laser fluorescence; NaF, sodium fluoride; SBS, shear bond strength; SMH, surface microhardness; T_0, baseline; T_1, post-cycling or post-lesion assessment; T_2, post-treatment assessment.



Table 2. Preventive effects of Aloe vera gel and 5% sodium fluoride varnish on enamel surface microhardness and laser fluorescence

A. Descriptive outcomes

Parameter	**Group A: Control (n = 15)**	**Group B: *Aloe vera* (n = 15)**	**Group C: 5% NaF (n = 15)**
Baseline microhardness (VHN)	329.99 ± 14.06	326.34 ± 18.47	332.91 ± 13.98
Final microhardness (VHN)	213.61 ± 17.20	268.46 ± 21.69	310.25 ± 14.20
Microhardness change (VHN)	−116.38	−57.88	−22.66
Baseline laser fluorescence	8.20 ± 1.20	7.57 ± 2.04	7.53 ± 1.61
Final laser fluorescence	17.91 ± 1.39	11.39 ± 2.57	9.07 ± 2.60
Laser fluorescence change	+9.71	+3.82	+1.54

B. Baseline-adjusted comparisons of final microhardness

Comparison	**Adjusted mean difference (VHN)**	**95% CI**	**Holm-adjusted *P***
B − A	+57.66	47.65 to 67.66	<0.001
C − A	+94.39	84.40 to 104.38	<0.001
C − B	+36.74	26.62 to 46.85	<0.001

Overall ANCOVA: $F(2, 41) = 185.53$; $P < 0.001$; $R^2 = 0.911$.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; NaF, sodium fluoride; SD, standard deviation; VHN, Vickers hardness number.

Notes: Values in Part A are mean ± SD, except changes, which represent differences between group means. Positive adjusted differences in Part B indicate higher final microhardness in the first group. ANCOVA was adjusted for baseline microhardness. Laser fluorescence outcomes are descriptive; the reported ANCOVA applies only to microhardness.



Table 3. Shear bond strength of orthodontic brackets following preventive interventions

A. Descriptive outcomes

Group	n	SBS (MPa), mean ± SD
A: Control	15	8.06 ± 1.53
B: *Aloe vera*	15	8.25 ± 1.54
C: 5% NaF varnish	15	6.20 ± 1.73

Overall one-way ANOVA: $F(2, 42) = 7.47$; $P = 0.0017$.

B. Tukey's post hoc comparisons

Comparison	Mean difference (MPa)	95% familywise CI	Adjusted *P*
B − A	+0.18	−1.24 to 1.61	0.9479
C − A	−1.86	−3.29 to −0.44	0.0076
C − B	−2.05	−3.47 to −0.62	0.0032

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; MPa, megapascal; NaF, sodium fluoride; SBS, shear bond strength; SD, standard deviation.

Notes: Mean differences are calculated as the first group minus the second group, using the underlying data rather than the rounded descriptive means. Confidence intervals and *P* values were adjusted for multiple comparisons using Tukey's method. The absence of a significant difference does not establish equivalence.

Table 4. Therapeutic effects of Aloe vera gel and 5% sodium fluoride varnish on enamel surface microhardness and laser fluorescence following artificial lesion induction

A. Descriptive outcomes

Parameter	**Group D: *Aloe vera* (n = 15)**	**Group E: 5% NaF (n = 15)**
Baseline microhardness (VHN)	333.59 ± 18.27	332.91 ± 18.28
Post-lesion microhardness (VHN)	218.64 ± 24.43	219.68 ± 20.63
Final microhardness (VHN)	265.59 ± 32.95	285.89 ± 25.35
Microhardness loss during induction (VHN)	−114.95	−113.23
Microhardness recovery (VHN)	+46.94	+66.21
Baseline laser fluorescence	6.58 ± 1.76	7.25 ± 1.53
Post-lesion laser fluorescence	15.49 ± 2.78	15.69 ± 3.71
Final laser fluorescence	10.65 ± 3.74	8.57 ± 4.83
Fluorescence reduction during treatment	4.84	7.12

B. Between-group comparisons of therapeutic changes

Outcome	**Mean difference (E – D)**	**95% CI**	**t(df)**	***P***
Microhardness recovery (VHN)	+19.27	8.50 to 30.04	3.67 (28)	0.0010
Laser fluorescence reduction	+2.28	0.50 to 4.05	2.63 (28)	0.0138

Abbreviations: CI, confidence interval; NaF, sodium fluoride; SD, standard deviation; VHN, Vickers hardness number.

Notes: Values in Part A are mean ± SD, except changes, which represent differences between group means. Microhardness recovery was calculated as final minus post-lesion microhardness. Fluorescence reduction was calculated as post-lesion minus final fluorescence. Positive between-group differences indicate greater treatment-related change in the fluoride group. Statistical comparisons were based on individual specimen-level change scores.

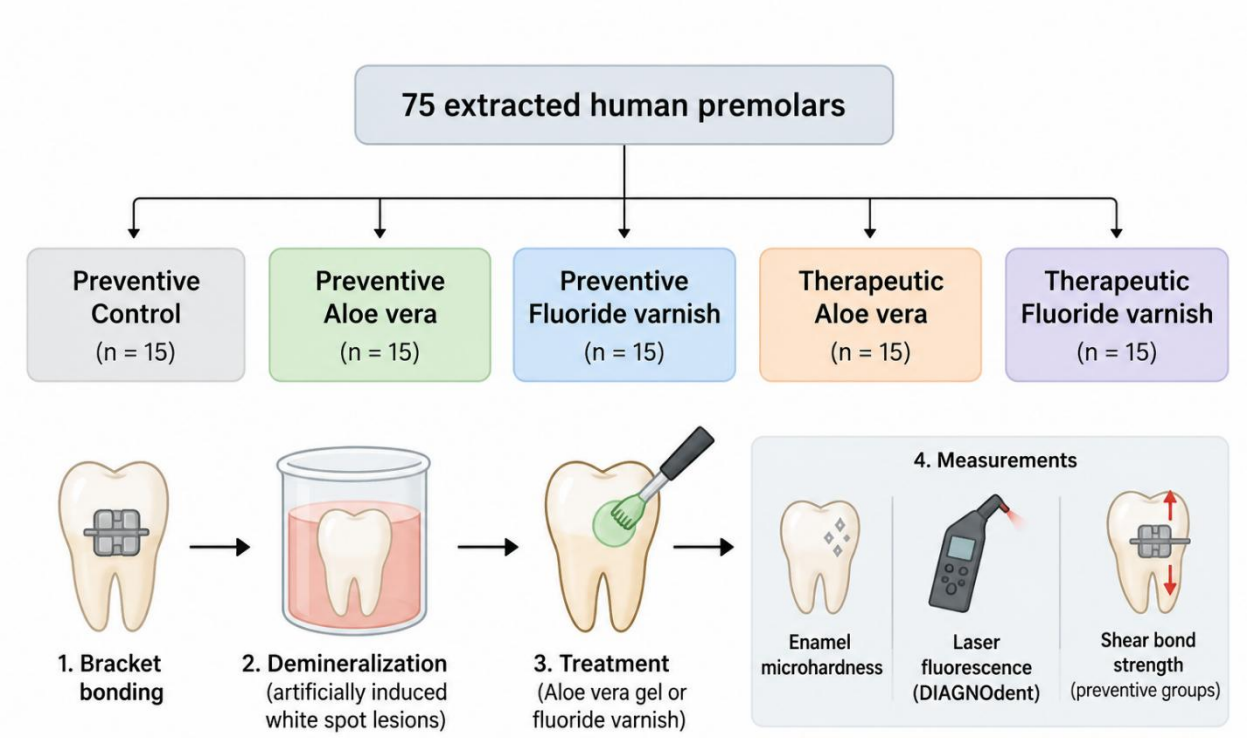


Figure 2. Preventive effects on enamel surface microhardness and laser fluorescence.

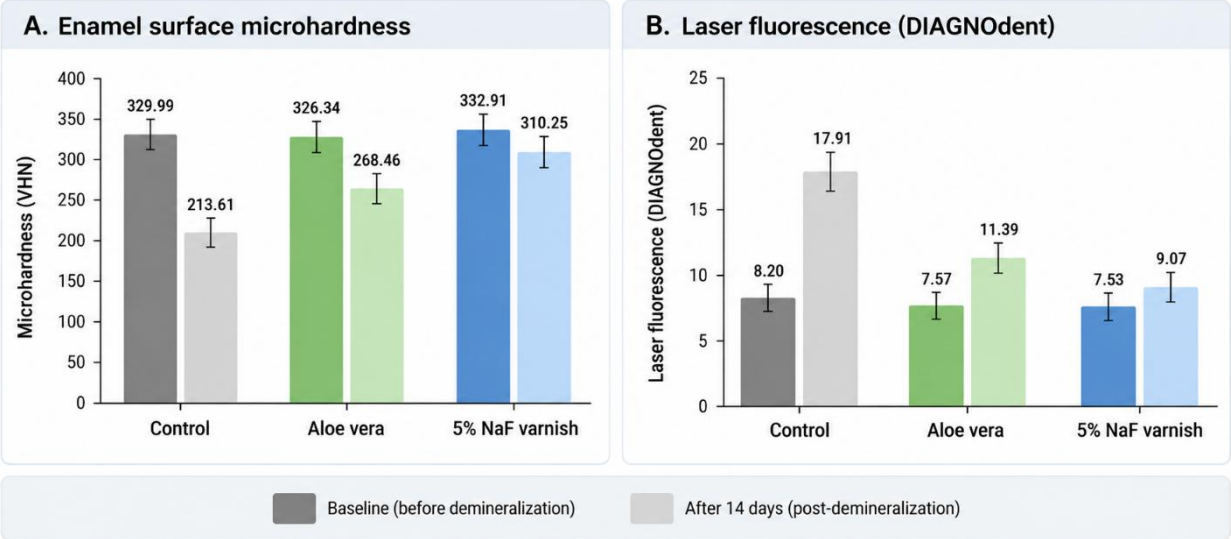


Figure 3. Therapeutic effects on enamel surface microhardness and laser fluorescence.

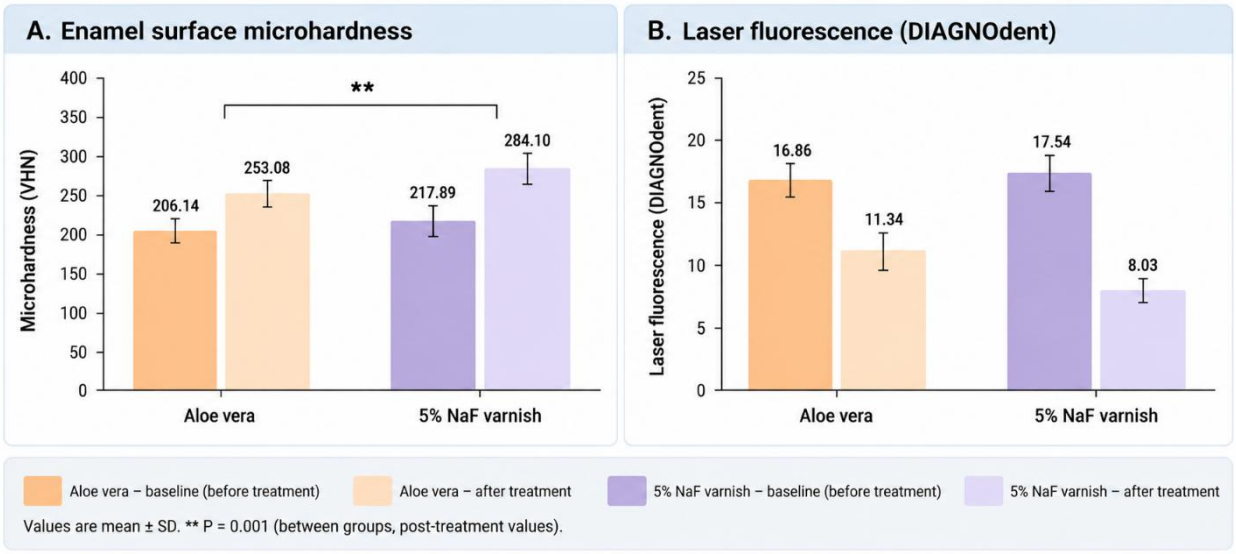


Figure 4. Shear bond strength of orthodontic brackets in the preventive groups.

