ORIGINAL ARTICLE **/** *ARTICLE ORIGINAL*

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COMPARATIVE EVALUATION OF SURFACE CHANGES AND BOND STRENGTH OF MICROABRASION BLEACHED ENAMEL BEFORE AND AFTER REMINERALIZATION WITH NANO-HYDROXYAPATITE PASTE: IN VITRO STUDY

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Objectives: Comparative evaluation of surface changes and bond strength of Microabrasion Bleached enamel before and after Remineralization with Nano-hydroxyapatite paste, an In vitro study.

Methods: Enamel samples were divided into - Group I - Conventional bleaching (HB), Group II - Conventional bleaching with remineralization (HB+N-Hp), Group III - Microabrasion-assisted bleaching(M+HB), and Group IV - Microabrasion-assisted bleaching with remineralization(M+HB+N-Hp). Surface roughness was measured using a profilometer. Samples were stored in artificial saliva for two weeks, then bonded with nanofilled composite resin and subjected to shear bond strength (SBS) analysis.

Results: Group IV (M+HB+N-Hp) showed higher surface roughness than Groups I (HB) and II (HB+N-Hp), while Group II had significantly lower surface roughness compared to Groups III (M+HB) and IV. In terms of shear bond strength (SBS), Group III exhibited significantly higher SBS than Group I, whereas Group II showed significantly reduced SBS compared to Group I. All values were statistically significant.

Conclusions: Remineralization with N-Hp reduces the surface roughness caused by bleaching and microabrasion, potentially decreasing bacterial biofilm adherence. Despite increased surface roughness correlating with better bond strength, microabrasion-assisted bleaching followed by remineralization and direct composite veneering after two weeks is a promising minimally invasive treatment for severe tooth discolorations. Further studies should explore varying frequencies and concentrations of N-Hp application.

Keywords: Tooth Bleaching, Tooth Remineralization, Enamel Microabrasion, Composite Resin, Bond Strength

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Conflicts of interest:

The authors declare no conflicts of interest.

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ÉVALUATION COMPARATIVE DES CHANGEMENTS DE SURFACE ET DE LA FORCE D'ADHÉSION DE L'ÉMAIL BLANCHI ASSISTÉ PAR MICRO-ABRASION AVANT ET APRÈS REMINÉRALISATION AVEC DE LA PÂTE NANO-HYDROXYAPATITE: ÉTUDE IN VITRO

Objectifs: Évaluation comparative des modifications de surface et de la résistance au collage de l'émail blanchi assisté par microabrasion avant et après reminéralisation avec une pâte de nanohydroxyapatite : Étude in vitro

Méthodes: Des échantillons d'émail ont été divisés en quatre groupes : Groupe I - Blanchiment conventionnel (HB), Groupe II - Blanchiment conventionnel avec reminéralisation (HB+N-Hp), Groupe III - Blanchiment assisté par microabrasion (M+HB) et Groupe IV - Blanchiment assisté par microabrasion avec reminéralisation (M+HB+N-Hp). La rugosité de surface a été mesurée à l'aide d'un profilomètre. Les échantillons ont été conservés dans de la salive artificielle pendant deux semaines, puis collés avec une résine composite nanocomposée et soumis à une analyse de la résistance au cisaillement (SBS).

Résultats: Le groupe IV (M+HB+N-Hp) a montré une rugosité de surface plus élevée que les groupes I (HB) et II (HB+N-Hp), tandis que le groupe II présentait une rugosité de surface significativement plus faible par rapport aux groupes III (M+HB) et IV. En termes de résistance au cisaillement (SBS), le groupe III a présenté une SBS significativement plus élevée que le groupe I, tandis que le groupe II a montré une SBS significativement réduite par rapport au groupe I. Toutes les valeurs étaient statistiquement significatives.

Conclusions: La reminéralisation avec N-Hp réduit la rugosité de surface causée par le blanchiment et la microabrasion, ce qui pourrait diminuer l'adhérence du biofilm bactérien. Bien que l'augmentation de la rugosité de surface soit corrélée à une meilleure résistance au collage, le blanchiment assisté par microabrasion suivi de la reminéralisation et du collage direct de résine composite après deux semaines constitue un traitement prometteur et peu invasif pour les décolorations dentaires sévères. Des études supplémentaires devraient explorer les fréquences et les concentrations variables de l'application de N-Hp.

Mots clés: Blanchiment des dents, reminéralisation des dents, microabrasion de l'émail, résine composite, force de liaison

Introduction

Addressing aesthetic concerns is a significant issue for patients and poses a challenge for dentists [1]. Many appealing smiles are affected by discoloration or staining, which can be localized to individual teeth or widespread. Isolated yellow, brown, or white areas on otherwise normal enamel are common. Advances in materials and techniques now effectively eliminate or conceal these issues. Treatment options vary depending on the severity of enamel stains, ranging from invasive ceramic veneers to abrasive chemical treatments. Despite the growing desire for flawless smiles, financial constraints influence treatment choices, making less invasive and cost-effective options such as bleaching, micro-abrasive treatments, and composite resin restorations are more common [1].

Enamel micro-abrasion (M) involves eliminating the porous enamel surface and trapped stains by gently rubbing the affected teeth with an acidic gel (such as 37% phosphoric acid or 15% hydrochloric acid), an abrasive substance (pumice), and a low-speed handpiece [2].

Microabrasioned teeth may appear darker due to the see-through enamel exposing the dentin color, justifying the use of a bleaching gel afterward. Bleaching involves applying chemical agents (like hydrogen peroxide or carbamide peroxide) to oxidize the pigments causing the staining [2]. Vital bleaching can be performed using either in-office or at-home techniques. This process can be expedited by applying heat or light to the bleaching agent, enhancing the chemical's effectiveness [3].

Many authors have concluded that bleaching causes alteration of enamel surface, increases porosities and decreases its microhardness [3, 4].

Bleaching combined with microabrasion can reduce enamel thickness and increase the penetration of oxygen free radicals into deeper layers, resulting in dental hypersensitivity [4].

Researchers also found out that this increase in enamel surface roughness leads to increase in enamel's susceptibility to bacterial biofilm adhesion and eventually increases the chances of recurrent staining. Hence, leading to failure of the treatment [5, 6].

To prevent this, various re-mineralization strategies are now being followed [7, 8].

Applying remineralizing agents directly to the affected area or incorporating them into bleaching gels has been found to reduce the surface roughness and decrease the biofilm adherence to the enamel surface. Remineralizing agents, such as nano-hydroxyapatite (N-Hp) helps in repairing the micro - defects of the enamel surface created by microabrasion and bleaching thereby, decreasing the hypersensitivity [8].

To the best of our knowledge, no study has been conducted where microabrasion assisted bleached enamel has been remineralized and checked for the surface roughness and its bond strength.

Hence, the aim of this study was to evaluate and compare surface effects and bond strength of Microabrasion assisted Bleached enamel surface before and after Remineralization with N-Hp.

Materials and Methods

Preparation and Selection of Teeth

The sample size was calculated and obtained using "Open-Epi Software" with and an alfa error of 5%, a Power of 80%, a SD of 6 and an expected reaction of 8. The calculation showed a minimum sample of 40.

Twenty teeth were collected (Figure 1-B) based on the inclusion and extrusion. Inclusion criteria included teeth with sound enamel, with no h/o bleaching and teeth extracted for orthodontic purpose whereas exclusion criteria included carious teeth, cracked teeth, teeth undergone prior bleaching, hypocalcified teeth, teeth with erosion and developmental anomalies, teeth with carious lesions and deciduous teeth.

The teeth were then cleaned using ultrasonic scaler, autoclaved and stored in 0.5% thymol solution.

All the teeth were thoroughly examined under the microscope (EX-TARO 300, Carl Zeiss, United States) for cracks. Teeth were then sectioned mesio-distally to obtain two enamel surfaces per tooth, thus total of 40 enamel samples were obtained.

Each sectioned tooth was embedded in cold cure resin cylinders (DPI RR) (1.5 cm wide by 1.5 cm high) with the enamel exposed. (Figure 1-C)

Randomization sequence generation-

Each sample was given a unique number. The allocation of the sample in each group was done using a computer-generated random sequence table. This was done using Sequentially numbered, opaque, sealed envelope (SNOSE) technique and concealed. The sample were then randomly divided into four groups $(n=10)$

GROUP I (Conventionally bleached enamel): (n=10)

According to manufacturer's instructions, the contents of bleaching gel (SDI Pola OfficeTN) were mixed and applied on clean dried enamel using a brush applicator till it reached a uniform consistency, after which a generous layer of the gel was placed and kept undisturbed for 8 minutes. (Figure-1D&E)

 Then, the bleaching agent was removed via suction. This constituted 1 bleaching cycle. This cycle was repeated twice for 20 minutes. Following the final application, the gel was removed with a suction and then rinsed using distilled water.

GROUP II (Conventional bleaching with remineralization): (n=10)

Similar to GROUP I, bleaching was performed. For remineralization, Dente 91 toothpaste, which contains nano-hydroxyapatite paste was applied to each sample using a micro brush applicator tip. As recommended by the manufacturer, this 1mm

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thick layer of N-Hp paste remained in contact with the sample surface for a period of 2mins, during which it was agitated and then was washed off with distilled water. (Figure 1-H)

GROUP III (Microabrasion assisted bleaching): (n=10)

For microabrasion, PREMA microabrasion kit was used. The samples were air-dried using a three-way syringe. According to manufacturer's instructions, a disposable curved dispensing tip was attached to the PREMA syringe, and a 1mm layer of PREMA slurry was applied to each sample. (Figure 1-F)

 Using a contra-angle handpiece on a slow-speed micromotor with a specially designed mandrel tip, the slurry was compressed against the sample surface for 60 seconds to initiate micro reduction, followed by rinsing. (Figure 1-G)

Immediately following microabrasion, bleaching was performed similar to Groups I and II.

GROUP IV (Microabrasion assisted bleaching post remineralization): (n=10)

Here, Microabrasion and bleaching were conducted as in Group III, followed by remineralization as performed in Group II.

Figure1. A: Armamentarium used; B: 20 extracted premolars selected based on inclusion criteria; C: The samples were cut in half mesiodistally and embedded in cold cure resin cylinders such that enamel remains exposed; D: The bleaching agent was mixed and; E: applied on to the samples according to manufacturer's instructions. Such cycles were repeated twice for each sample.; F&G: For Groups III and IV, prior to bleaching, microabrasion was performed according to the manufacturer's instructions.; H: For remineralization in Groups II and IV, DENTE 91 toothpaste was applied and agitated according to manufacturer's instructions; I: Surface roughness evaluation under a portable profilometer, SJ-210 (Mitutoyo, Kanagawa, Japan) with a static load of 5 N and a speed of 0.05 mm/s; J: Samples stored in containers containing artificial saliva for a period of 2 week prior to bonding procedure; K: bonding procedure was performed according to manufacturer's instructions followed by incremental composite buildup; L: Shear bond strength (SBS) analysis under Universal Testing Machine (UTM) (Instron, LLOYD instruments LRX, UK) with a knife-edge shear blade, at a crosshead speed of 1 mm/min.

Blinging

The assessor of the outcome (the laboratory technicians evaluating the samples) were blinded after assignment to intervention.

Surface roughness testing

The samples were evaluated using a calibrated SJ-210 profilometer (Mitutoyo, Kanagawa, Japan). Standard roughness (Ra) was measured with a 5 N load and 0.05 mm/s speed, using a 0.25 mm cutoff to filter surface ripples. (Figure-1I)**.** Three measurements were taken at various positions on each surface, and the arithmetic mean was computed.

After analysis, the samples were stored separately in four containers with artificial saliva (Wet Mouth Liquid, ICPA HEALTH PRODUCTS LTD) for 2 weeks, with daily changes of the saliva. (Figure 1-J)

Shear bond strength

After two weeks, the samples were rinsed, dried, and etched with 37% phosphoric acid (PRIME Dental, Pvt Ltd) for 15 seconds. A bonding agent (Single Bond Universal Adhesive, 3M ESPE, Germany) was applied following the manufacturer's guidelines. Plastic cylindrical tubes (3x4mm) were placed on each sample, filled with composite resin in increments, and cured for 20 seconds at 850mW/cm² (WOODPECK-ER). (Figure 1-K)

 The tubes were gently removed using tweezers. The samples then underwent shear bond strength (SBS) testing with a knife-edge shear blade in a universal testing machine (UTM) (Instron, LLOYD Instruments LRX, UK) at a crosshead speed of 0.5 mm/minute. (Figure1-L)

Statistical Analysis

Surface roughness and shear bond strength were analyzed, with descriptive statistics for each group reported as the mean and standard deviation (SD). The analysis was conducted using SPSS (Statistical Package for Social Sciences) ver-

Figure 2. Showing mean surface roughness across groups

sion 19. One-way analysis of variance (ANOVA) and the post hoc Pair wise comparison was done using Tukey's post hoc test. ANOVA is a versatile and powerful statistical technique, and the essential tool when researching multiple groups or categories. The one-way ANOVA can help you know whether or not there are significant differences between the means of your independent variable. Tukey's post hoc test was used to calculate significance of differences between pairs of group means.

All statistical analyses were performed at a significance level of p < 0.05.

Results

Surface Roughness

The standard roughness (Ra) was measured under a portable profilometer, SJ- 210 (Mitutoyo, Kanagawa, Japan) using a static load of 5 N and a speed of 0.05 mm/s. Ra representing the arithmetic mean between the recorded peaks and valleys, while using a 0.25 mm cutoff to filter out any surface ripple.

The Mean surface roughness of Group I (bleached enamel) was 0.68±0.37, in Group II(bleached enamel post remineralization) was 0.81±0.26. In Group III (microabrasion assisted bleached enamel) and

IV (microabrasion assisted bleached enamel post remineralization) the surface roughness was 1.25±0.87 and 1.17±0.49 respectively (Figure 2).

Pair wise comparison was done using Tukey's post hoc test showed statistically high significant difference when Group 4 was compared with Group 1 and 2 respectively (p<0.001). A statistically high significant difference in surface roughness was observed when Group II was compared with Group III and IV respectively (p<0.001).

There was no statistically significant difference between I and II and in between Group III and IV in the surface roughness (p>0.05).

Shear Bond Strength

The shear bond strength was conducted in the Universal testing machine (UNITEST 10, ACME Engineers, India.) Shear loads were applied at the adhesive-enamel interphase at a crosshead speed of 0.5 mm per minute until debonding occurred. The maximum load required to debond was recorded in Newtons and was then converted to Megapascals (MPa).

The mean values and the standard deviation of Shear bond strength for each is shown in figure 3. Mean shear bond strength of Group I

(I) Group	(J) Group	Mean Difference	p value	95% Confidence Interval	
		$(I-J)$		Lower Bound	Upper Bound
Group I	Group II	-0.13	.599	-1907	.8612
	Group III	-0.57	.164	.1588	.215
	Group IV	-0.49	.0786	.1580	.4145
Group II	Group I	0.13	$< 0.001*$	-0657	.1907
	Group III	-0.44	.908	-0.213	-0.477
	Group IV	-0.36	.112	.0205	.4770
Group III	Group I	0.57	$< 0.001*$	-4152	.1588
	Group II	0.44	$< 0.001*$	$-.4777$.2213
	Group IV	0.08	1	-1290	.1275
Group IV	Group I	0.49	$< 0.001*$	-0.4145	-1580
	Group II	0.36	$< 0.001*$	$-.4770$.2205
	Group III	-0.08	.431	-1275	.1290

Table 1. Pairwise comparison of surface roughness between Group I, II, III and IV.

* The mean difference is significant at the 0.05 level.

(bleached enamel) was 13.50±2.32, in Group II (bleached enamel post remineralization) was 7.68±2.14. In Group III (microabrasion assisted bleached enamel) was 14.00±2.44 and IV (microabrasion assisted

bleached enamel post remineralization) was 16.08±3.85 respectively

Pair wise comparison was done using Tukey's post hoc test showed statistically high significant differ-

ence when Group IV was compared with Group 1, 2 and 3 respectively (p<0.001). A statistically high significant difference in shear bond strength was observed when Group 3 was compared with Group 1 and Group 1 with group 2 respectively (p<0.001) (Table 2).

There was no statistically significant difference between Groups I and II and in between Groups III and IV in the shear bond strength $(p>0.05)$.

Discussion

In minimally invasive dentistry, combining bleaching with direct composite veneering is the most economical and conservative method for addressing tooth staining, as veneers alone often can't fully mask intrinsic stains [9].

In the present study, microabrasion assisted bleaching with and

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Table 2. Pairwise comparison of shear bond strength between Group I, II, III and IV.

* The mean difference is significant at the 0.05 level.

without the use of remineralizing agent, i.e. N-Hp was done.

Microabrasion removes the discoloration by abrading the tooth surface microscopically. Here 6.6% hydrochloric acid slurry containing silica carbide particles was used for microabrasion. This aids in surface discoloration eradication while simultaneously creating a smoother surface by eliminating micro-pores. This process enhances the brightness and reflectivity of the enamel, thereby improving its aesthetic appeal [9, 10].

With combining microabrasion to bleaching, the efficacy of bleaching is increased, along with more penetration of hydrogen peroxide into the deeper layers of enamel. As studied by Costa et al. which suggests that microabrasion, combined with bleaching and minor enamel/

dentin adjustments, significantly affects hydrogen peroxide (HB) penetration.[10] This diffusion aspect is clinically important, especially for thin enamel, which may allow greater peroxide diffusion through dental tissues [10].

HB concentrations can vary from 15 - 40%, thus, at higher concentrations such as 35% - 40% has shown better esthetic results and is more potent as shown in literature [11].

Here, 35% hydrogen peroxide bleaching (HB) with 8 minutes of application per cycle (three cycles) [Pola Office, SDI] was used to bleach the enamel surface.

HB stands as the most formidable and potent oxidizing agent. Upon interaction with dental tissues, it dissociates into reactive oxygen and free radicals, which then permeate the dental tissues and oxidize the pigments [11].

When such techniques are employed clinically, there are high chances of dentin hypersensitivity [12].

Presently, nano hydroxyapatite paste (N-Hp) is being preferred as a desensitizing and remineralizing agent because of the hydroxyapatite particles in nanometerised size, which have been proven to be highly potent [13].

This N-Hp is commercially available as a toothpaste (Dente 91 toothpaste), which contains nano-hydroxyapatite, lactoferrin, xylitol, aqua, sorbitol, silica, glycerin, PEG 8, SLS, xanthan gum, fragrance, titanium dioxide, sodium saccharin, sodium benzoate.

Dente 91 not only remineralizes but also has antifungal and biocompatible properties. Lactoferrin, with its strong iron affinity, sequesters this nutrient from harmful microor-

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ganisms, disrupting their growth. This salivary lactoferrin is crucial in protecting enamel and oral microbes, promoting optimal oral hygiene [13].

In a study by Huang S. et. al. (2011), they evaluated the effectiveness of N-Hp in remineralizing early carious lesions.

This research indicated that the efficacy of remineralization is heightened when the size of the hydroxyapatite (HA) particle is decreased to the nanometric range [14].

Studies indicate that bleaching alters surface morphology and microabrasion reduces enamel thickness. N-Hp was used to mitigate these changes, reducing hypersensitivity and remineralizing enamel post-treatment [15, 16].

Due to its surface remineralization properties, adding an apatite coating to teeth, N-Hp is preferred over other desensitizing agents for treating hypersensitivity. N-Hp binds to demineralized surfaces, forming micro clusters that create an even apatite layer, restoring enamel morphology and reducing surface roughness [15].

In this study, highest mean surface roughness was observed in G III (M+HB) and least was seen with G I (HB). This is observed since M increases the enamel's susceptibility to demineralization post-bleaching, resulting in more pronounced surface changes and greater enamel permeability [16].

Group IV (M+HB+N-Hp) showed reduced surface roughness as compared to G III (M+HB), though this was not statistically significant. Though not statistically significant, this result was in accordance with the study by Khouroushi et. al. 2015, where it was observed that remineralizing agents such as N-Hp when applied along with bleaching agent causes significant reduction in surface roughness. This can be observed since the N-Hp helps in repairing the micro- defects of the enamel surface created by microabrasion and bleaching thereby, decreasing surface roughness [17].

Similarly, in a study by Lima De (2017) the use of N-Hp prior to bleaching gel application retained enamels microhardness and surface texture [18].

Group IV (M+HB+N-Hp) showed significantly higher roughness compared to G I (HB) & II (HB+N-Hp). This finding was similar to the study performed by Franco et. al 2015 where, enamel surface roughness was significantly influenced by microabrasion, regardless of being combined with dental bleaching [19].

While N-Hp aids in the restoration of the enamel surface, the alterations caused by the combination of microabrasion and bleaching are so significant that it becomes impractical to entirely reverse these changes.

Similarly, Group II (HB+ N-Hp) showed significantly lower surface roughness compared with G III(M+HB) & IV (M+HB+N-Hp), This aligns with Tong et al.'s study and may be due to the different stain-removal actions of HB and M. Hydrochloric acid decalcifies both enamel and the stains within it (McEvoy, 1989) [20].

 Mixed with pumice, it significantly reduces surface enamel. Similar findings were observed by Moharam LM et al. (2021), where N-Hp reduced surface irregularities [21].

Various studies suggest bonding to a bleached surface should be done after 1-2 weeks to ensure the removal of oxygen free radicals, which may interfere with the photopolymerization of composite restorations [22, 23].

In a 2015 study by Mirhashemi et al., laser-assisted and conventional bleaching of enamel were compared with varying delays before bonding metallic brackets. SBS testing showed reduced strength when bonding immediately after bleaching. However, a 1-hour delay after laser-assisted bleaching (30%

hydrogen peroxide) and a 1-day delay after conventional bleaching (40% hydrogen peroxide) restored SBS. Therefore, in the present study, bonding was performed after 2 weeks, with samples stored in Wet Mouth liquid (ICPA Health Products Ltd) [24].

For the bonding procedure, the etch and rinse method was used. Studies suggest this method offers better bond strength to enamel than dentin, making it more predictable for composite restorations [25].

In this study, all the groups showed statistically significant values where the lowest mean SBS was of Group II (HB+N-Hp) and highest was of Group IV (M+H- $B+N-Hp$).

Since microabrasion alters the surface morphology and makes enamel permeable, there is better penetration of HB. In addition, it increases the surface roughness of enamel, as seen with our above observations. This increase in the surface roughness practically leads to larger surface area for bonding to occur.

In a study by Rahmanpanah S. et. al. (2023) they evaluated the SBS of orthodontic brackets with enamel remineralized with various concentrations of H-Np. They found that H-Np at lower concentrations tends to increase the SBS however when the concentration is increased, the SBS gradually reduces [26].

Group III (M+HB) showed significantly higher SBS as compared to Group I (HB). The microabrasion method enhances the roughness of the enamel surface, irrespective of the utilization of either 18% or 35% phosphoric acid, or 6.6% hydrochloric acid with abrasive [27].

Since the collaboration of HB with M results in further heightened roughness, the surface area for bonding is greater, thus, giving better SBS [28].

Group II (HB+N-Hp) showed significantly reduced SBS compared to Group I (HB). N-Hp (nano-hy-

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droxyapatite) can repair the small imperfections in enamel caused by bleaching. This restoration of the enamel surface reduced the surface irregularities thus, prevents a strong bond with composite restorations [29].

There was no statistically significant difference in SBS observed between Group III (M+HB) and Group IV (M+HB+N-Hp). In Group IV a 1mm thick layer of N-Hp was applied to each sample and agitated for a period of 2 minutes. However, this duration proved to be inadequate in fully reversing the alterations induced by HB and M.

Conclusion

Within the limitations of the study, it can be concluded that N-Hp remineralization after microabrasion-assisted bleaching reduces the surface roughness of enamel caused by bleaching and microabrasion, which helps to prevent post-operative sensitivity clinically and bacterial biofilm adherence.

For SBS lowest mean SBS was of Group II (HB+N-Hp) and highest was of Group IV (M+HB+N-Hp). Thus, microabrasion-assisted bleaching after remineralization will improve the bond strength of direct composite veneering or any esthetic restorative procedure in management of discoloration.

Limitations

The limitations of this study include a small sample size (n=10 per group), which restricts the generalizability of the findings. Being an in vitro study, it does not fully replicate the oral environment. Laboratory conditions does not account for patient behavior, oral hygiene, and diet variations. The accuracy of profilometer and shear bond strength tests could vary, and findings require clinical validation.

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