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Shear Bond Strength of Light Cured Resin Composite to Bleached Enamel Pre-Treated with Probiotic

Panneerselvam Nithyashri[1], Shekar Shobana[2*], Sundaresan Balagopal[3], Sebatni Anisha[4], Sarath Sarathy[4] and Murugan Nivetha[1]

[1]Student, Department of Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Chennai – 600127, Tamil Nadu, India
[2]Reader, Department of Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Chennai – 600127, Tamil Nadu, India; drshobana@tagoredch.in
[3]Head of the Department, Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Chennai – 600127, Tamil Nadu, India
[4]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Chennai – 600127, Tamil Nadu, India

Abstract

Aim: To assess the Shear Bond Strength (ShBS) of light cured composite resin to bleached enamel pre-treated with probiotic. **Materials and Method:** 40 extracted permanent maxillary central incisors teeth were obtained, decoronated, mounted on resin blocks and grouped into four namely Group 1: Resin Composite restoration done without bleaching (positive control), Group 2: Vital bleaching is done with 40% hydrogen peroxide for 20 minutes followed by composite restoration (negative control), Group 3: Composite restoration delayed by 4 weeks after vital bleaching, Group 4: Composite restoration on bleached enamel pretreated with probiotic for 60 minutes. All the specimens were conserved for one day (24 hours) in deionized water and subjected to analysis for ShBS using cross head speed of 0.5 mm per minute. **Statistical Analysis:** Data was tabulated on excel sheet and analyzed statistically using SPSS software. **Results:** One way ANOVA statistical test showed a statistically significant difference in the mean ShBS values between the groups. Group 1 (positive control), Group 3 (delayed bonding), and Group 4 (probiotic) had considerably higher values of mean ShBS than Group 2 (immediate bonding). **Conclusion:** Probiotic pre-treatment on bleached enamel was able to effectively reverse the effect on ShBS of enamel treated with bleaching agent.

Keywords: Bleaching, Composite, Enamel, Probiotics, ShBS, Universal Testing Machine

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1. Introduction

Tooth discoloration and stains on the teeth can happen for a variety of reasons. Tooth staining affects the confidence of an individual and has a negative impact on their psychology. The causes for tooth discoloration are manifold and usually classified as intrinsic or extrinsic[1]. Extraneous chromogens are deposited on the tooth surface causes extrinsic staining. Intrinsic discoloration occurs when the chromogens are deposited within the dentin[1].

Tooth discoloration can be managed by various dental procedures such as tooth bleaching, macroabrasion, microabrasion, veneering and use of porcelain crowns. Tooth bleaching is a frequently sought after aesthetic dental procedure to tackle the problem of tooth discoloration[2].

Bleaching is a redox process which uses a chemical agent, to oxidize the organic pigmentation present within the tooth. Bleaching agents like hydrogen peroxide (H_2O_2), carbamide peroxide, sodium perborate, have been used for whitening the teeth. These oxidizing agents, after diffusion into the enamel and dentin,

*Author for correspondence

disintegrates to produce unstable, oxygen free radicals. These residual oxygen free radicals attack the organic pigmented molecules and reflects less light, thus creating a "whitening effect" in the tooth[3].

In some cases, bleaching procedures may have to be followed by a resin composite restoration when the degree of tooth lightening obtained is not considered adequate by the patient. It has been reported that the bond between the bonding agent and the tooth surface is hampered post-bleaching. The explanation for the reduction in bond strength of the restorations placed immediately after bleaching may be attributed to alterations in the chemical structure of the enamel as pore formation and subsequent entrapment of oxygen residues, which interferes with the light-curing of the dental adhesives and interferes with enamel resin tag formation quantitatively and qualitatively[4].

Usually, the bonding protocol is delayed by 2-4 weeks to revert back the bond strength. This delay in bonding after bleaching may not be acceptable to some patients[5]. Previous techniques, like treating the enamel with alcohol before the application of composite, removal of enamel superficial layer, use of adhesive containing organic solvents, and use of antioxidants can improve the bond strength to bleached enamel[5].

The application of an antioxidant agent is one of the methods suggested to reverse the compromised bond strength of the resin to bleached enamel [5]. Antioxidants terminate the chain reactions by removing free radical intermediates and inhibit further oxidation reactions by neutralizing free radicals. Sodium ascorbate[6], superoxide dismutase[7], cranberry extract[8], green tea[5] α-tocopherol[6] and other antioxidants have also been analysed and compared to each other in exploration for the most adequate protocol to reverse the reduction in bond strength of restorations in bleached teeth[4].

Probiotics have been considered as an emerging source of effective antioxidants[9]. Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". They are the mixture of several species or a single microorganism containing *Lactobacillus, Escherichia, Bifidobacterium, Bacillus, Enterococcus and Streptococcus* species. Probiotics have been used for the management and prevention of various medical ailments and have been shown to be effective in treating a variety of clinical conditions like infantile diarrhea, necrotizing enterocolitis, antibiotic-associated diarrhea, *H. pylori* infections, surgical infections, etc[9].

According to a systematic review by Seminario-Amez *et al.*, probiotics were found to be beneficial in maintaining oral health and reducing the colony-forming units of the oral microflora. Probiotics have been tried for the management of dental caries and periodontal diseases[10]. Probiotics for clinical usage are available as beverages, prebiotic fibers, milk additives, yogurts, cheese, bio-drinks, dietary supplements such as powder, capsules, etc[10].

According to Wanget *et al.*, probiotics regulate signalling pathways and attune the redox status of the host, chelate metal ions, and possess intrinsic antioxidant mechanisms[11]. In 2011, Chin-Feng Liu suggested that *Lactobacillus exopolysaccharides* exhibit significant *in vitro* antioxidant activity[12]. Cuevas-Gonzalez concluded that the cytoplasmic content of *Lactobacillus paracaseiis* a promising source of metabolites with antioxidant properties that may help reduce the acrylamide-induced oxidative stress in human erythrocytes[13]. This evidence from the literature prompted us to tap the properties of probiotics to explore the bond strength reversal potential of light-cured composite resin to bleached enamel.

Pre-clinical assessment of the adhesive properties of the composite to teeth can be assessed by both macro and micro bond tests. Since adhesive joints in practical settings are exposed to both shear and tensile loads, it's important to assess these parameters to evaluate the strength of adhesive joints[14]. Thus, in the present study, *Lactobacillus paracasei,* which is proven to have the strongest antioxidant effects amongst the other *Lactobacillus* species[13] has been used to assess the reversal of Shear Bond Strength (ShBS) of composite resin to bleached enamel. To the best of our knowledge, no study has been published in the literature so far utilizing probiotics for assessing the reversal of bond strength to bleached enamel.

2. Materials and Methods

This *in-vitro* study was performed at Tagore Dental College and Hospital, Rathinamangalam, Chennai after approval from the Institutional Ethics Committee [IEC/TDCH/161/2021].

2.1 Inclusion Criteria

Forty extracted permanent maxillary central incisor teeth with intact enamel surfaces.

2.2 Exclusion Criteria

Fractured teeth and teeth with dental caries, attrition, abrasion, erosion, hypoplastic teeth, and previously restored or endodontically treated teeth were excluded from the study.

2.3 Specimen Preparation

The specimens were prepared by a single calibrated operator. All the specimens were decoronated by sectioning the roots 2mm below the cemento-enamel junction by using a slow-speed diamond disc under copious water spray. The crowns were then embedded in an autopolymerizing acrylic resin block (3.0 x 3.0 x3.0 cms) with an incisal edge facing upwards.

40 Specimens were randomly divided into four groups.

Group 1 (n = 10): Positive control group (Composite restoration on unbleached enamel)

Group 2 (n = 10): Negative control (Composite restoration done immediately after bleaching procedure)

Group 3 (n = 10): Composite restoration delayed 4 weeks after bleaching

Group 4 (n = 10): Study group (Immediate composite restoration after bleaching followed by probiotic treatment for 60 minutes)

Groups 2,3 and 4 received bleaching procedure as follows.

2.4 Bleaching Procedure

Bleaching was done with 40% Hydrogen peroxide (Opalescence boost, Ultradent Products, Inc, South Jordan UT) for 20 minutes following the manufacturer's instructions. A small amount of bleaching gel was dispensed on the labial surface and the gel was completely rinsed off with water spray for 60 seconds after 20 minutes.

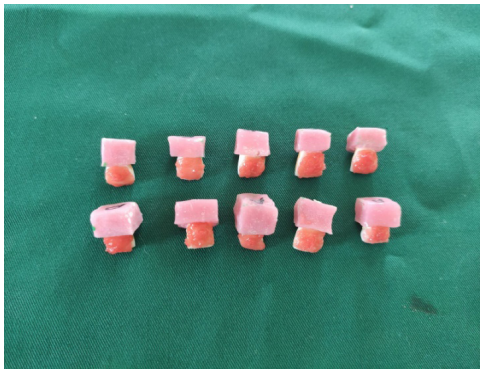


Figure 1. Application of bleaching agent on the labial enamel of the prepared samples.

Group 1 (positive control) and Group 2 (negative control) received composite restoration immediately. Group 3 received composite restoration after four weeks of storage in distilled water. Group 4 received composite restoration after immersing the bleached specimens in probiotic [YAKULT- Non-fat probiotic milk with live and active *Lactobacillus paracaseishirota,* Yakulthonsha Inc, Japan] for 60 minutes.



Figure 2. Immersion of the bleached samples in probiotic solution.

2.5 Bonding Procedure

Etching of the specimens was done with 37% phosphoric acid gel (Eco-Etch gel, *Ivoclar vivadent,* Te-Econom plus) for 15 seconds followed by water rinsing for 20 seconds and air dried for 5 seconds. Adhesive (*Ivoclar vivadent* Te-economy Bond) was then applied, gently air-thinned, and cured for 10 seconds. Teflon tube 2 mm in diameter and 2 mm in height was used to prepare samples of standardized sizes. The Teflon tube was placed on adhesive-applied enamel surfaces. Light-cured Composite resin (*Ivoclar vivadent*, Te-Econom plus Composite) was then filled into the tube as a single increment and light-cured (Bluphase, Dentsply) for 40 seconds. After polymerization, the Teflon molds were removed and specimens were placed in distilled water for 24h. After 24 hours, the specimens of groups 1, 2 and 4 were tested for ShBS. Bonding in Group 3 (delayed bonding) was done after four weeks as explained above and subjected to ShBS test.

Specimens were mounted in the jig of Universal Testing Machine (Instron India Private Limited) for the shear bond test, and the shear force was applied using a steel knife edge shearing rod at the adhesive interface

until failure occurred. The load was applied to the adhesive interface, as close as possible to the surface of the substrate at a crosshead speed of 0.5 mm/min. ShBS values were expressed in MegaPascal (MPa).

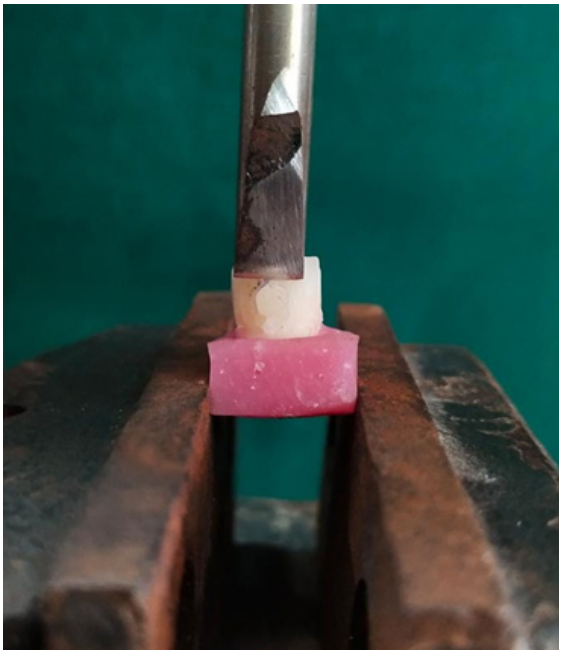


Figure 3. Samples subjected to shear loading on universal testing machine.

3. Results

Statistical analysis using one-way ANOVA revealed that there was a significant difference in the mean ShBS values between the groups (p = 0.001) (Table 1) (Figure 4). Post hoc test revealed that the mean ShBS values of Group 1, Group 3 and Group 4 were significantly higher than Group 2 (p ≤ 0.001) (Table 2). However, there was no statistically significant difference in the mean ShBS values between Group 1 (10.87 MPa), Group 3 (14.115 MPa) and Group 4 (13.77 MPa). (Group 3 > Group 4 > Group 1).

Table 1. Mean of shear bond strength between the groups

Groups	Mean ShBS	Standard Deviation	F	P Value
Group 1 (Positive Control)	10.870	6.466	13.977	0.001*
Group 2 (Immediate Bonding)	0.010	0.010		
Group3 (Delayed Bonding)	14.115	1.475		
Group 4 (Probiotic)	13.770	9.015		

p < 0.05 (* denotes statistically significant difference)

* The mean difference is significant at p < 0.05 level.

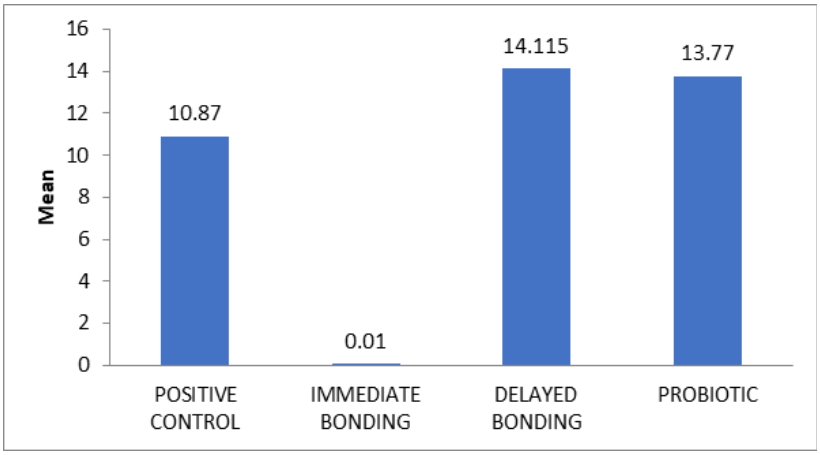


Figure 4. Mean of ShBS between the groups..

Table 2. Post hoc tukey test comparing the difference in mean ShbS between the groups

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	10.859500*	2.502721	.001	4.11910	17.59990
	3	-3.245500	2.502721	.571	-9.98590	3.49490
	4	-2.900600	2.502721	.656	-9.64100	3.83980
2	1	-10.859500*	2.502721	.001	-17.59990	-4.11910
	3	-14.105000*	2.502721	.000	-20.84540	-7.36460
	4	-13.760100*	2.502721	.000	-20.50050	-7.01970
3	1	3.245500	2.502721	.571	-3.49490	9.98590
	2	14.105000*	2.502721	.000	7.36460	20.84540
	4	.344900	2.502721	.999	-6.39550	7.08530
4	1	2.900600	2.502721	.656	-3.83980	9.64100
	2	13.760100*	2.502721	.000	7.01970	20.50050
	3	-.344900	2.502721	.999	-7.08530	6.39550

4. Discussion

A substantial reduction in bond strength of composite resins to bleached enamel has been noted against unbleached enamel[7]. Akin *et al.,* concluded that 38% hydrogen peroxide adversely affected the bond strength of the orthodontic brackets to bleached enamel[15]. A reduction in enamel-resin bond strength could be caused by factors such as calcium loss, decreased microhardness, and biochemical alterations in the organic portion of the substrate[7]. Some studies suggest that bleached enamel and dentin have decreased bond strengths due to a redox potential imbalance created by the bleaching agents. Residual oxygen from the bleaching agent causes disruption of resin polymerization. Unshared electrons in free radicals possess high energy and react quickly with ambient oxygen to generate reactive oxygen species[7]. The storage period of 24 hours - 3 weeks was found to eliminate the chemical changes caused by the incorporation of peroxide ions[7]. Such a condition may not be suitable for patients with immediate aesthetic requirements. Various experiments were conducted in order to restore the diminished bond strength so that immediate aesthetic adjustments could be carried out employing adhesive restoration. Treatment of bleached enamel with an antioxidant demonstrated an instantaneous increase in bond strength values to some extent, however, the other

techniques yielded mixed results in terms of salvaging bond strength values[16].

Antioxidants are substances that inhibit the degree of oxidation and cause radicals to be converted into inactive derivatives[17]. Previously, several antioxidants such as Sodium Ascorbate, alpha-tocopherol, proanthocyanidins, pine bark extract, cranberry extract, superoxide dismutase Sodium bicarbonate, catalase, green tea, catalases were used to reverse bond strength[5,7].

Pathak *et al.,* concluded that applying the application of sodium ascorbate hydrogel for 1 minute in two phases was effective in counteracting the detrimental effects of bleaching agents on enamel ShBS[18].

In 2013, Thapa *et al.* inferred from their study that following 10% carbamide peroxide bleaching, applying 10 and 25% sodium ascorbate solutions for 10 minutes, and also 25% alpha-tocopherol solution, improved the ShBS of composite resin to enamel significantly[6]. According to Kavitha *et al.,* Sodium Ascorbate, Alpha-tocopherol and Super Oxide Dismutase can effectively reverse the bond strength of bleached enamel[7].

A lot of research has been directed to finding prudent antioxidants from bio-resources to replace synthetic antioxidants.

Results of the current study revealed that Group 1 (positive control), Group 3 (delayed bonding), and Group 4 (probiotic) had considerably higher mean ShBS than Group 2 (immediate bonding). Delaying the bonding procedure by 14 days might be a practical solution for reversing the effects of internal bleaching[19]. The antioxidant-treated group had significantly higher bond strength which is consistent with the fact that using antioxidants immediately after bleaching can neutralize leftover oxygen and reverse bond strength reduction[20]. As previously discussed, bleaching affects the biochemical properties of enamel which will adversely affect the quality of the bonding substrate. The very poor bond strength of the immediate bonding group could be because the superadded free radical complicates adhesion. The delayed bonding group had the highest mean ShBS values. This is because of the decomposition of the free radicals on storage for four weeks. The test group pre-treated with probiotics had mean ShBS values comparable to the delayed bonding group. The mechanism behind this phenomenon can be hypothetically stated thus*:* *L. casei* spp inhibited lipid peroxidation and maintained the activity of superoxide dismutase and catalases[21]; counteracted the effects of aflatoxin[22]; minimizes the oxidative stress in rat models[23]. According to an *in vivo* animal study by Kapila *et al.* Wistar albino rats were given dietary supplements of *Lactobacillus paracasei* extracts and found to have *L. casei* spp the maximum antioxidant property as witnessed by biochemical assays amongst 12 tested strains[24].

Group 1, which received composite restoration on untreated enamel surfaces had mean ShBS values lower than delayed bonding and probiotic pre-treated group even though the difference was statistically insignificant. The mechanism behind higher bond strength values in delayed and probiotic group in comparison with the positive control remains unsubstantiated and is the scope for future research. This study was undertaken to evaluate the ShBS of composite resin to bleached enamel using probiotics as an antioxidant. ShBS tests have been widely employed, owing to their relative ease when compared to tensile bond strength tests, which can be difficult to align without causing detrimental stress distribution. Further studies that analyze the adaptability of the composite resin to probiotic pre-treated enamel at microscopic levels will be helpful to translate the results of this study into clinical practice. Shear tests provide several advantages, including ease of specimen preparation and a simple test technique[25]. This study is novel in that no evidence in the literature has resorted to using probiotics as an antioxidant for the reversal of bond strength of bleached enamel. The limitation of the present study is that specimens were pretreated with probiotic solution for 60 minutes which is practically difficult in a clinical setup also further studies need to be conducted adjusting the antioxidant pre-treating time and results must be co-related. In the present study, use of probiotics has been effective in reversing of bond strength to bleached enamel. Probiotics used in the present study is an edible, non-toxic antioxidant and hence absolutely safe to use. This research opens newer avenues for advanced clinical research and exploring its further applications. This study has assessed immediate bond strength after probiotic application. Assessing the delayed bond strength could be a future scope of the study.

5. Conclusion

Within the limitations of the present study, it can be concluded that probiotic pretreatment was able to effectively reverse the bond strength of bleached enamel.

The mechanism of action of probiotics, and its effect on other bonding parameters like tensile bond strength need to be assessed to draw reliable conclusions.

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