



Original Article

Evaluation and comparison of shear bond strength and adhesive remnant index score in brackets bonded with probiotics impregnated orthodontic adhesive and commercially available orthodontic adhesive- An in vitro study

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Abstract:

Aim: White spot lesions are one of the main drawbacks of fixed orthodontic mechanotherapy. Probiotic bacteria have been included in mouthwashes and lozenges to check the activity of *S.mutans* on surface enamel. Aim of the present study was to evaluate and compare shear bond strength and adhesive remnant index scores of orthodontic brackets bonded with probiotic impregnated (*Lactobacillus rhamnosus*) orthodontic adhesive and conventional light cure orthodontic adhesive.

Materials and methods: In this in-vitro study, two groups were tested in one group brackets were bonded to extracted maxillary premolars using probiotic impregnated composite and in the other with conventional light cure composite resin. Shear bond strength was assessed using universal testing machine. Adhesive remnant index scores were assessed on the same samples.

Results: Shear bond strength of the new composite resin was significantly decreased when compared to conventional light cure composite ($p=0.001$). There was no significant difference in ARI scores ($p=0.51$) between the groups.

Conclusions: The shear bond strength of the novel probiotic impregnated composite was significantly reduced. Though there was no significant variation in ARI scores when compared to control, avenues to improve the shear bond strength should be assessed.

Key words- Probiotic impregnated composite resin, white spot lesions, *Lactobacillus rhamnosus* GG, *Streptococcus mutans*.

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INTRODUCTION

White spot lesions are due to subsurface enamel demineralization and is one of the consequences of fixed orthodontic treatment if oral hygiene maintenance is poor.^[1] These lesions occur around the brackets with or without cavity formation. Prevalence of White spot lesion in orthodontic patients ranges from 2% to 96%.^[2] The labio-gingival areas of maxillary laterals are most susceptible to white spot lesions and the maxillary posteriors are least susceptible.^[2]

In the orthodontic patients, *Streptococcus mutans* present in the plaque ferments carbohydrate and releases acid by products which leads to enamel demineralization and white spot lesion.^[3] Mechanical plaque control methods like brushing and flossing can help improve patient's oral hygiene. Fluoride enhances enamel resistance by calcium fluoride formation, when acidity of plaque biofilm increases due to the activity of oral microflora there is release of fluoride.^[4] There are various modes in which fluorides can be administered, they are fluoride mouth rinses, varnish, toothpaste, luting cements, bonding agents, pits and fissure sealants, fluoride releasing elastomers, fluoride containing antibacterial adhesives. In recent times, the use of casein phosphopeptides amorphous calcium phosphate(CPP-ACP) has shown promise in the reduction of white spot lesion formation.^[4]

Antiseptic mouthwashes were shown to effectively reduce streptococcus mutans count. Lasers have been found to enhance the microhardness of enamel making it resistant to acid dissolution caused by cariogenic bacteria.^[5] Management of white spot lesions during retention period is done by a standard protocol of prescribing the use of CPP-ACP and fluoride. A solution of 10% carbamide peroxide has been found to camouflage white spot lesion without any change in the mechanical and chemical properties of enamel.^[6] Micro abrasion, by means of abrasive slurry of 6% hydrochloric acid is effective if the deepest point of the lesion is 0.2 mm or less.^[7] Erosion-infiltration is a recent method which uses low viscosity resin.⁷ Natarajan et al studied the effect of adding methacryloyloxydodecylpyridinium bromide (MDPB) in conventional glass ionomer cement (GIC) and found that there was reduction in the adherence of *S.mutans* thus increasing the resistance of conventional GIC to *S.mutans*.^[8]

Though numerous regimens are prescribed to promote remineralization after orthodontic treatment, they are all heavily reliant on patient's compliance. Probiotics have been shown to reduce white spot lesions by significantly reducing the *Streptococcus mutans* count in the plaque.^[9-11] In an earlier study, fluoride and calcium were incorporated in resin composite and leaching of these ions reduced enamel demineralization.^[12] In the present study, probiotic bacteria–*Lactobacillus rhamnosus* GG, have been impregnated in light cure composite resin used to bond orthodontic brackets to enamel and effect of the same on the mechanical properties of the resin composite have been studied. Aim of the study was to evaluate and compare shear bond strength and adhesive remnant index score (ARI) among brackets bonded with probiotic impregnated orthodontic adhesive and conventional light cured orthodontic adhesive.

Null hypothesis as stated is “there is no difference in the bond strength and adhesive remnant index score between brackets bonded using probiotic impregnated light-cure composite resin and conventional available light cure composite.”

MATERIALS AND METHODS:

SAMPLE SIZE DETERMINATION:

Sample size for the study was calculated using G power software (version 3.1.9.2), for a power of 85% and alpha error of 5%, sample size determined was 31 per group. Sixty-two extracted maxillary premolar teeth were taken for Experimental (Group I) and control group – conventional light cure orthodontic adhesive (Orthofix, Anabond Stedman Pharma Research pvt LTD)(Group II).

A novel composite resin (group 1) was manufactured by adding Lactobacillus rhamnosus GG at 2.05% to 20-40% of resin matrix, 60-80% of fillers, erythritol 1.50% and whey protein 3.5% (Anabond Stedman Pharma Research PVT LTD, Chennai, India).

Patency of Lactobacillus rhamnosus GG in the polymerized composite

Patency of the bacteria Lactobacillus rhamnosus GG in the polymerized resin (Group 1) was checked by bonding brackets with the probiotic impregnated composite on extracted premolar teeth. The teeth were placed in sterile saline for two days, later transferred to BHA broth and stored in it for 7 days the broth was then cultured in De Man, Rogosa and Sharpe (MRS) agar. Growth of Lactobacilli rhamnosus GG was seen in the culture medium. Hence it was concluded that the lactobacilli impregnated in the composite was patent.

Determination of bond strength and Adhesive Remnant Index Scores:

After preserving in a disinfectant solution of 0.5% chloramine-T at 6°C, the teeth were embedded in gypsum type III dental stone. 37% phosphoric acid was applied to etch the enamel surface and rinsed after 30 seconds. After application of primer, brackets were positioned on the tooth surface after marking the long axis. Orthodontic adhesive was cured using LED curing light for 40 seconds (Bluphase, Ivoclar). Bonding was done by the same operator. In control group, brackets were bonded using conventional orthodontic adhesive and experimental group brackets were bonded with probiotic impregnated orthodontic adhesive.

Specimens were stored in water (37°C) in a thermostatically controlled tub. A shear force from the incisal-to-cervical direction was applied at the bracket-tooth interface by a chisel-shaped rod fixed to the crosshead of a universal testing machine at speed of 1 mm/min. The load at failure was recorded in Newtons and converted to megapascals (MPa) to obtain shear bond strength (SBS) by dividing the shear force (Newton) by the area of the bracket base (square millimeters).^[13]

Adhesive Remnant Index Score

The adhesive remnant index was studied for the 31 samples under probiotic impregnated orthodontic adhesive (Group1) and was compared with the 31 samples control group bonded with conventional light cured orthodontic adhesive (Group 2). The ARI scores were evaluated in both the groups after testing shear bond strength with Instron testing machine using the following criteria.^[14]

1. No remnant on the tooth
2. 1-25% adhesive left on the tooth
3. 26-50% adhesive left on the tooth
4. 51-75% adhesive left on the tooth
5. 76-99% adhesive left on the tooth
6. All adhesive left on the tooth with impression of the bracket mesh

RESULTS:

Shapiro-Wilk test was done to evaluate the normality of the data. The test shows that the data was normally distributed. Unpaired t test was used to compare the shear bond strength between the conventional and probiotic impregnated composite. Chi square test with Yates correction was used to assess ARI score.

Unpaired t test (Table-1) was performed to compare the shear bond strength between Group-I (probiotic impregnated orthodontic adhesive) and group-II (conventional light-cured orthodontic adhesive). It was found that there was statistically significant reduction ($P < 0.001$) in shear bond strength in probiotic impregnated orthodontic adhesive when compared to the conventional group. This indicates that the new resin with *Lactobacillus* has decreased SBS when compared to the commercially available light cured composite resin.

Table 1: Unpaired t test for comparison of shear bond strength between experimental and control group in MPa.

	Groups	Mean	S.D	Std. error mean	T	P value		Mean Diff:	95% Confidence Interval	
						One-Sided p	Two-Sided p		Lower	Upper
SBS	Control	8.58	3.0	.54	11.8	.001	.001	6.6	5.5	7.7
	Probiotic	1.97	.9	.16						

ARI index of the samples was observed after testing SBS in both the groups. Chi square test (Table-2) was used to compare the ARI index between the group-I probiotic impregnated orthodontic adhesive and group-II conventional orthodontic adhesive. It was found that there was no statistically significant difference ($P = 0.51$) in Adhesive remnant

index in brackets bonded with probiotic impregnated composite when compared with the conventional group. This indicates that the new resin with Lactobacillus has similar ARI index to the available conventional resin.

Table – 2. Chi-Square test with yates’ correction for comparison of ARI score between experimental and control group.

		Adhesive Remnant Index Score					Chi-square	P value
		1	2	3	4	5		
Group	Control	1	1	9	7	13	3.295	0.51
	Probiotic	1	3	12	8	7		

DISCUSSION:

White spot lesions (WSL) are subsurface enamel demineralization that are milky white patchy and discernible clinically. Other names for white spot lesions are Incipient lesions and Surface-softened defect.^[15,16]

Probiotics are defined as living non-pathogenic microorganisms which when prescribed in adequate amount are beneficial to the host.^[9] According to Elie Metchnikoff, longevity of Bulgarian population was because of consumption of lactic acid containing fermented products.^[17]

Bafna et al observed that there was statistically significant reduction in the Streptococcus mutans count after consumption of yogurt containing Lactobacillus acidophilus and Bifidobacterium.^[18]

Yousuf et al found out that use of probiotic mouthwashes reduced plaque accumulation in children.^[19] Alanzi et al showed that the consumption of probiotic lozenges everyday showed reduction in the microbial count.^[20]

The survival of Lactobacillus rhamnosus GG in acidic environment is significantly improved when compared to other species in the same environment.^[21] Hence in this study. Lactobacillus rhamnosus GG was impregnated in the conventional composite.

Jose et al proved that the use of Probiotic toothpaste had efficiently reduced the Streptococcus mutans growth in the plaque.^[9] Similar research by Cildir, Villavicencio et al involving inclusion of probiotics in the diet in form of milk or yogurt was found to reduce S.mutans count in the experimental group when compared to the control.^[11,22] Research on this topic till date has relied on patient’s compliance to either consume probiotic rich food for stipulated period of time or use probiotic toothpaste.

Remineralization of enamel and dentin requires certain ions like calcium and fluoride, incorporation of these ions into composite showed promising effects on the remineralization of enamel and dentin. These ions present in the composite showed leaching effects.^[12,23] In the present study, probiotic was impregnated in the composite resin which obviates the need for dependence on patient’s compliance.

Test for Shear bond strength (SBS) was performed in both probiotic impregnated composite (Group I) and conventional composite (Group II) one week after bonding brackets on extracted human premolars. It was observed that the shear bond strength of conventional composite (Group II) was significantly higher when compared to the probiotic impregnated composite (Table 1). The disadvantage of this novel probiotic impregnated composite is reduced shear bond strength.

In this study it was found that impregnation of probiotics in the composite reduced the shear bond strength. It was observed during shear testing that the new composite resin peeled off in layers. This may be due to the plasticizing effect of the probiotics incorporated. Hence, the methods of increasing the shear bond strength of the novel probiotic impregnated composite requires additional research. ARI score was assessed in the debonded brackets after testing for shear bond strength. It was observed that there was no significant difference in the ARI score between the two groups (Table-2). Thus, the ARI score of the novel probiotic impregnated composite is similar to the conventional composite. The majority of ARI score of both the groups were 3 and 5, which indicates that the primary fracture occurs at the adhesive- bracket interface. Thus, cohesive strength of the composite and the adhesive bond to the enamel is more than the adhesive bond to the bracket base. Pseiner et al evaluated the ARI score of the fluoride releasing composite and found it to be higher than that of conventional composite.^[23] The SBS of novel probiotic impregnated resin was reduced when compared to the conventional composite but the ARI score of the probiotic impregnated composite is similar to the conventional composite.

CONCLUSION:

The shear bond of the novel probiotic impregnated composite is significantly reduced. Though, there was no significant increase in bracket failure or variation in ARI scores when compared to control, avenues to improve the shear bond strength should be assessed.

SOURCE OF FUNDING:

Nil

CONFLICT OF INTEREST:

The authors have no conflicts of interest to declare.

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