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Streptococcus mutans levels in patients who received orthodontic brackets bonded using probiotic impregnated resin composite – a randomized clinical trial

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ABSTRACT

To evaluate the effect of impregnating an orthodontic resin composite with probiotic bacteria (*Lactobacillus rhamnosus* GG) on the levels of *Streptococcus mutans* (*S. mutans*) in patients undergoing orthodontic treatment. Thirty patients were randomly selected and allotted by block randomization to two groups: an experimental group, who received brackets bonded with probiotic impregnated resin composite and a control group, who received brackets bonded with conventional light cure resin composite. Plaque samples were collected before (ET0 and CT0) and two months (ET1 and CT1) after bonding. Levels of *S. mutans* were assessed using the colony count method. Two months after bonding of the brackets, the *S. mutans* levels had decreased with statistical significance in the experimental group ($p = 0.001$), but not in the control group ($p = 0.137$). Impregnation of resin composite with probiotic bacteria for the purpose of preventing formation of white spot lesions on enamel holds promise. Long-term evaluation would be necessary to provide confirmatory results.

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Probiotic impregnated resin composite; white spot lesions; *Lactobacillus rhamnosus* GG; *Streptococcus mutans*

Introduction

Fixed orthodontic appliance therapy is wrought with many challenges, one of them being the maintenance of proper oral hygiene. Accumulation of excessive plaque not only jeopardizes the periodontal health but also demineralizes enamel, leading to formation of white spot lesions which are caused by dissolution of calcium salts in the enamel subsurface by the action of acidogenic bacteria like *Streptococcus mutans* (*S. mutans*) [1].

Jing et al. studied the effect of orthodontic appliances on oral microbial flora and concluded that there was a significant increase in the level of *S. mutans* in saliva of patients undergoing orthodontic treatment [2]. White spot lesions (WSL)appear as white patches or lines on the enamel surface especially at mesio and disto-gingival aspect of the orthodontic bracket, due to increased retention of plaque. The lesions might lead to cavitation of the enamel surface if left unattended. Tufekci studied the formation of WSL in orthodontic patients and found that there was a steep increase in the incidence of WSL with 46% of patients developing at least one such lesion during treatment [3]. Mirzhahi et al. concluded that there was both an increase in the prevalence of WSL and in the opacity index of enamel following orthodontic treatment [4]. They were most seen afflicting maxillary lateral incisors followed by canines, premolars and central incisors [5].

There are various ways of preventing the formation of WSL and one of them being the use of dentifrices with constituents like CPP-ACP, fluoride mouth rinses, or topical fluoride application [6–15]. Addition of supplements containing probiotic bacteria like curd, lozenges or use of a probiotic dentifrice, that are organic in nature instead of chemical, to counter the multiplication of acidogenic bacteria in the oral cavity has been gaining momentum in recent times [16–21].

The inhibition of pathogenic oral bacteria by probiotic microbes such as *Lactobacillus rhamnosus* could take place through any of the following three mechanisms; by competing for adhesion sites thereby preventing pathogenic proliferation, by consumption of the nutrients and growth factors thereby exhausting

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the pathogenic oral bacteria, or by inhibition of collagenases, reducing the mechanism of inflammation and enhancement of the immune response [22].

Researchers have prescribed probiotic containing edible substances like fruit yogurt, icecream, lozenges, or mouth rinses, to study participants in the quest of finding out if there was a reduction in the levels of *S. mutans* and have come up with conflicting results [16–21,23,24]. Yousuf et al. found that probiotic mouth rinses reduced gingival and plaque scores in children [25]. Many species of lactobacillus have also been studied but survival of *L. rhamnosus* GG was found to be better in acidic environment, in the presence of sugar which they metabolize, when compared to others [24].

Though research in the prevention of WSL by utilizing probiotic bacteria holds promise, all the methods are heavily dependent on patient compliance. The idea of this proof-of-concept study was novel in that the probiotic bacterium *L. rhamnosus* GG was impregnated in a light cured resin composite. Therefore, the aim of this study was to evaluate the number of colony forming units(CFU) of *S. mutans* in patients undergoing orthodontic treatment who received brackets bonded using probiotic impregnated resin composite before and after two months of bonding and to compare with control subjects who received orthodontic brackets bonded with conventional light cure resin composite.

The null hypothesis tested was that there would be no significant difference in the levels of *S. mutans* colonies between the experimental and the control group two months after bonding of the brackets.

Materials and methods

Sample size was calculated using Gpower software for this randomised parallel arm clinical study and for a power of 95% and alpha error of 5% the total sample size (n) arrived at was fifteen. Sample size was increased to thirty to compensate for drop out and error occurring by chance (15/group) [21].

Participants, eligibility criteria, settings

In this single blinded study, patients were allocated using block randomization in the allocation ratio of 1:1 following block randomization with a block size of 4. Thirty patients who reported to the Department of Orthodontics, were randomly selected based on the following criteria (1) patients (19–32 years) requiring orthodontic treatment (2) complete eruption of permanent teeth excluding the third molars (3) patients with no untreated primary caries and (4) patients who did not require premolar extraction for the purpose of orthodontic correction.

Subjects with potential confounders like smoking habit, diabetes mellitus, female patients on contraceptive pills, patients under antibiotic regimen, which might interfere with the oral microflora were excluded from the study. Subjects with profound gingival and plaque indices were also excluded from the study.

All thirty participants in the study were asked not to consume curd or, antibiotics and not to use fluoridated mouth rinses for 60 days but to use a tooth paste without fluoride. Though a split- mouth design is considered superior for clinical research in oral cavity, such a design was not used in the present study to prevent a cross-over effect of probiotic bacteria impregnated in the resin composite on control side. Baseline values of *S. mutans* levels were taken from both the experimental and the control group before bonding of the orthodontic brackets.

Composition of the resin composite

The resin composite was manufactured by adding *L. rhamnosus* GG to a conventional light cure resin composite (Orthofix, Anabond Steadman Pharma Research (P) Ltd) used for orthodontic bonding. The new resin composite contained 20–40% of resin matrix, 60–80% of fillers, 0.75% of erythritol, 1% *L. rhamnosus* GG and 1.75% whey protein. *Lactobacillus rhamnosus* GG was found to have better survival rate in acidic environment as compared to other probiotic microbes. Hence it was selected for the study [26]. Furthermore, Lactobacillus has been proven to be cultured better in the presence of a whey-protein supported medium [27]. The probiotic bacteria were dispersed along with the other fillers into the resin matrix through mechanical blending.

The viability of the impregnated probiotic was assessed by bonding brackets with the resin composite on extracted premolars, which were then stored in saline for a week for complete polymerization. The samples were cultured in De Man, Rogosa and Sharpe agar for two months. Growth of *L. rhamnosus* GG was seen in the culture medium thus confirming that the bacteria impregnated were viable.

Bonding procedure

Prior to bonding of the brackets, moisture control was achieved by a saliva ejector and cheek retractor,

Table 1. Demographic data with statistical analysis.

		Frequency			
Descriptive parameters	19–32 years	Experimental group	Control group	Statistical analysis	*p* Value
Mean age		21.46 ± 1.56	21.26 ± 1.33	Independent *t*-test	0.77
Gender	Male	8	7	Chi-square test	1
	Female	7	8		

and to improve isolation, cotton rolls were placed in the sulcus. Etching was performed with 37% orthophosphoric acid (Eazetch, Anabond Steadman Pharma Research (P) Ltd) applied for 20 s. Teeth were then rinsed for 15 s and dried with compressed air. After application of primer (Orthofix, Anabond Steadman Pharma Research (P) Ltd), probiotic impregnated composite was used to bond each bracket in the experimental group and light-cured conventional resin composite (Orthofix, Anabond Steadman Pharma Research (P) Ltd) was used in the control group. Photopolymerisation was performed using an LED curing unit(Ivoclar Bluephase N MC). The polymerization time was 40 s, 20 s for each of the mesial and distal aspect.

Sample collection and processing

Plaque samples were collected before (ET0 and CT0) and two months (ET1 and CT1) after bonding of the brackets. Before bonding, samples (baseline) were collected from the 30 patients using the 4-pass technique from the labial surfaces of the following maxillary and mandibular teeth: right central incisor, right second premolar, left central incisor and left second premolar [21].

The collected samples were placed in separate Eppendorf tubes and were stored in a deep freezer at −80 °C until processing.

The samples were vortexed for 1 min for dispersion in saline and submitted to tenfold serial dilutions. They were then plated equidistantly on TYCSB agar (tryptone yeast extract cysteine agar with sucrose and without Bacitracin) and incubated at 37 °C for 2–3 days. The colony count was performed using a digital colony counter.

Statistical analysis

To analyze the data, SPSS software (IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp. Released 2015) was used and the significance level was fixed at 5% ($p = 0.05$). Shapiro-Wilk test was performed to evaluate the normality of data and it was found that the data for all the parameters studied were normally distributed. The levels of *S. mutans* of the experimental (E) and control (C) groups were statistically compared at baseline (T0) and after two months (T1) using unpaired *t*-tests. The levels of *S. mutans* count before and after bonding in each of the two groups were compared using paired *t*-tests.

Results

Table 1 shows the distribution of patients by age and gender. No statistically significant differences were found between the experimental and the control group with respect to age ($p = 0.77$) or gender ($p = 1$). Table 2 shows the results of inter and intra-group comparison for number of *S. mutans* colony forming units (CFU) in the plaque samples taken before (ET0 and CT0) and after two months (ET1 and CT1) of bonding the orthodontic brackets. At baseline, there was no statistically significant difference in the number of *S. mutans* CFU between the experimental and control group ($p = 0.726$). In the experimental group, there was a significant reduction in the number of *S. mutans* CFU, two months after bonding of the brackets ($p = 0.001$), whereas there was no significant reduction in the control group ($p = 0.137$). This meant that two months after bonding of the brackets (ET1 and CT1), the number of *S. mutans* CFU was significantly lowered in the experimental group than the control group ($p = 0.001$).

Discussion

Numerous studies have attempted to research methods to prevent the formation of white spot lesions through the leaching of ions like calcium and phosphate from Bioactive Glass(BAG), utilizing either the efficiency of amorphous calcium phosphate (ACP) impregnated in the resin, through fluoride releasing high filled resin composite, or through the antimicrobial efficacy of organo-selenium compounds around orthodontic brackets [28–32]. The present research was an attempt to test the efficacy of leached probiotics from a light cure resin composite on the number

Table 2. Comparison of the experimental (E) with control (C) group.

Unpaired *t*-test to compare *S. mutans* colony forming units at T0 in the two groups

						95% confidence interval		
N = 30	Mean ± SD (CFU)	STD error mean	*t*-Value	Mean diff	STD error mean difference	Lower	Upper	*p* Value
ET0	143.40 ± 31.87	8.23	0.35	3.4	9.60	−16.28	23.08	0.73
CT0	140 ± 31.57	4.95						

Unpaired *t*-test comparing *S. mutans* colony forming units at T1 in both the groups

ET1	37.33 ± 16.70	4.31	7.52	82.67	11.00	60.15	105.12	0.001
CTI	120 ± 39.17	10.14						

Paired *t*-test to compare *S. mutans* colony forming units at T0 and T1 in experimental group

							95% confidence interval		
N = 15	Mean (CFU)	Std. deviation	Std. Error Mean	Total mean	Std. deviation	Std. error mean	Lower	Upper	*p* Value
ET0	143.40	31.89	8.23	106.07	22.82	5.89	93.43	118.70	0.001
ET1	37.33	16.70	4.31						

Paired *t*-test to compare *S. mutans* colony forming units at T0 and T1 in control group

						95% confidence interval		
N = 15	Mean (CFU)	Std. deviation	Std. error mean	Std. error mean	*t*-Value	Lower	Upper	*p* Value
CT0	140	31.57	8.23	5.89	1.53	−6.81	46.81	0.137
CT1	120	39.17	10.11					

Unpaired *t*-tests were used to compare the *Streptococcus mutans* colony forming unit (CFU) among patients who received brackets bonded with probiotic impregnated resin composite and conventional resin composite at baseline (T0) and two months after bonding orthodontic brackets (T1). Paired *t*-tests were used to compare the *Streptococcus mutans* CFU before and two months after bonding brackets with probiotic impregnated resin composite and conventional resin composite respectively.

of CFUs of *S. mutans* in plaque around orthodontic brackets.

Nase et al. demonstrated that administration of dairy products containing *L. rhamnosus* reduced the risk of dental caries in subjects by reducing the number of *S. mutans* colonies [33]. Therefore in the present study, the probiotic bacteria Lactobacillus rhamnosous GG was impregnated in a light cure resin composite used to bond orthodontic brackets and its effect on *S. mutans* colonies in the plaque samples collected from teeth surrounding the brackets were studied. There was a positive inhibitory effect on the *S. mutans* level as indicated by the reduction in the number of colony forming units in the experimental group. The null hypothesis was thus rejected.

Researchers have tried to incorporate probiotic bacteria in the daily routine of subjects and gathered evidence of their effects on oral microflora. Jose et al. found a statistically significant reduction in the levels of *S. mutans* in patients who consumed both probiotic curd and used probiotic tooth paste when compared to control groups after 30 days of trial [21]. Cildir et al. advised their orthodontic patients to consume fruit yogurt containing Bifidobacterium animalis and observed *S. mutans* levels at four periods [18]. Bafna et al. prescribed probiotic yogurt to high caries risk individual [20]. Caglar et al. researched the effects of icecream containing *Bifidobacterium lactis*, probiotic lozenges on *S. mutans* levels in plaque of patients in two separate trials [17,24]. All these studies reported significant reductions in the levels of *S. mutans* in their experimental groups, which is in alignment with the results of the present study. The advantage of the present research design was that it did not rely on patient compliance for the intervention.

Conflicting results were recorded by Gizani et al. who studied the effectiveness of probiotic lozenges containing *Lactobacillus reuteri* in preventing white spot lesion formation. The authors reported no difference in the number of white spot lesions between a control group and an experimental group at the time of bracket debonding [16]. Possible reasons for the lack of effect could be the following discrepancies in methodology; the intended intervention was started only 6 months after bonding of the orthodontic brackets and white spot lesions were evaluated on photographs taken after debonding.

In the present study, samples taken from the control group showed a small reduction in the CFU count of *S. mutans*. This could be attributed to the anti-microbial properties of dental adhesives as concluded by Lapinska et al. or could be due to improved oral hygiene practice of patients after bonding of the orthodontic brackets [34]. The reduction was nevertheless not significant, which is in concordance with the result of Caglar et al. who found no significant difference in the *S. mutans* levels in the placebo group who consumed ice cream [23].

Though several studies point out the diverse nature of microbiota responsible for the formation of white spot lesions, the present research was confined to analyzing the variation in the levels of *S. mutans* alone because it appears to be the predominant microbe responsible for the initiation of caries [34,35]. Despite that some species of Lactobacillus have been proven to take part in the progression of decay [36]. The primary focus of this study was on establishing the antibacterial nature of the probiotic impregnated composite on *S. mutans*. Future studies should determine the effect of impregnation of probiotic bacteria in resin composite on other microbiota.

Conclusion

Bonding orthodontic brackets using a resin composite impregnated with probiotic bacteria reduced the levels of acidogenic streptococci in the dental plaque of patients two months after bonding. This promising result warrants a long-term evaluation to assess the actual prevention of white spot lesion formation.

Author contributions

Krishnaraj Rajaram: Conceptualization; Data curation; Formal analysis; Resources; Project administration; Supervision; Validation; Visualization; Roles/Writing – review and editing. Poornima. R. Jnaneshwar: Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Supervision; Validation; Visualization; Roles/Writing – original draft; Writing – review and editing. Azmina Idaayath: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Roles/Writing – original draft, funding – self funded. Ravi Kannan: Conceptualization; Data curation; Formal analysis; Resources; Project administration; Supervision; Validation; Visualization; Roles/Writing – review and editing.

Disclosure statement

There are no relevant financial or non-financial competing interests to report. All the authors report no conflict.

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