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Effect of Antimicrobial Varnish on Microbial Concentration Around Orthodontic Brackets"– An *Invivo* Study

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KEYWORDS

Demineralization, Whitespot lesions, Orthodontic brackets, Chlorhexidine-Thymol varnish, Microbial concentration, Antimicrobial activity

ABSTRACT:

Introduction: Fixed orthodontic appliances limit the ease of naturally occurring salivary cleansing and antimicrobial activity thereby promoting proportionate growth and agglomeration of aciduric bacteria with time, leading to formation of active white spot lesions.

Objectives: Preventing development of WSLs during orthodontic treatment has been attempted through various approaches

Methods: In this study, 4 groups with 10 subjects each were selected, and with split mouth design Chlorhexidine-Thymol antimicrobial varnish (Cervitec® Plus, Ivoclar Vivadent, Lischenstein)premixed with primer is applied on the premolars during bonding and its effect on the microbial composition and concentration around orthodontic brackets, is evaluated through microbial culture at 1st, 2nd, 3rd and 4th months after debonding.

Results: The result shows that within the controls, the microbial counts were found to be gradually increasing from a value of 5.9 ±1.3 (in 10^4) in group I to 14.9 ± 1.9 (in 10^4) in group IV while in the experimental sample, the count increase from a value of 1.2 ± 0.6(in 10^4) in group I to 13.3 ± 2.6 (in 10^4) in group IV.

Conclusions: It is concluded that the application of chlorhexidine-thymol antimicrobial varnish premixed with primer during orthodontic bonding showed effective reduction in the microbial counts in vivo, for the duration of four months, with proportionate decline in the antimicrobial effect with time.

1. Introduction

. Among the many orthodontic appliances, brackets are the paramount cause for enamel demineralization or white spot lesion formation because of their complex design, resulting in retention of food particles and dental plaque.[1] It is documented by Human Microbiome Database (HMD) that approximately 600 prokaryotic bacterial species comprise the plaque microflora and oral ecosystem.

Enamel demineralization is caused by the acids produced by mutans streptococci (MS), which are known to be the prime causative organisms of dental caries.

Composites, which are the predominantly used dental biomaterials for bonding orthodontic brackets to the tooth surface, are one of the sources for the accumulation of plaque and opportunistic bacteria as well.[2]

The complexity of the design of the components of fixed orthodontic appliances like the brackets, bands, and wires restricts the ease of naturally occurring salivary cleansing and antimicrobial activity thereby promoting proportionate growth and agglomeration of aciduric bacteria with time. This encourages the enamel demineralisation, formation of active white spot lesion (WSL) and caries, if ignored.[3]

Preventing development of WSLs during orthodontic treatment has been attempted through various approaches. Chlorhexidine is considered as the most potent antimicrobial agent against the oral

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microbes especially the mutans streptococci (MS). As compared to all other delivery methods of chlorhexidine, application in the form of varnish results in, enduring suppression of MS concentrations.[4] The amalgamation of orthodontic bonding materials with chlorhexidine varnish could play a crucial role in preventing demineralization and WSL's during orthodontic treatment.

The aim of this study is to evaluate the effect of Chlorhexidine-Thymol varnish (Cervitec® Plus, Ivoclar Vivadent, Lischenstein) on the microbial composition and concentration around orthodontic brackets, in vivo.

Objectives

Preventing development of WSLs during orthodontic treatment has been attempted through various approaches

Materials and methods:

This study includes 40 randomly selected patients irrespective of their gender and ethnic origin who were ready to begin orthodontic treatment. These subjects were divided into 4 groups (I, II, III, & IV) according to the debonding time interval, with 10 subjects in each group. Patients with systemic disorders, pregnancy, periodontitis, patients using antibacterial mouth rinses during the study period were excluded from the study.

A split mouth design where forty premolar brackets in the first quadrant served as experimental group with and forty premolar brackets in the second quadrant served as control group. In control sample bonding is done with the normal 3 step etch-prime- bond procedure. In the experimental group, premolars were etched, rinsed and dried similar to the control group but the primer used was a preparation of chlorhexidine-thymol antimicrobial varnish (Cervitec® Plus Antimicrobial varnish) mixed with Primer (Transbond XT, 3M UNITEK) at a ratio of 2:1. This combination was applied to the etched enamel surface and cured for 10 seconds, and then the brackets were bonded with adhesive, similar to control sample.

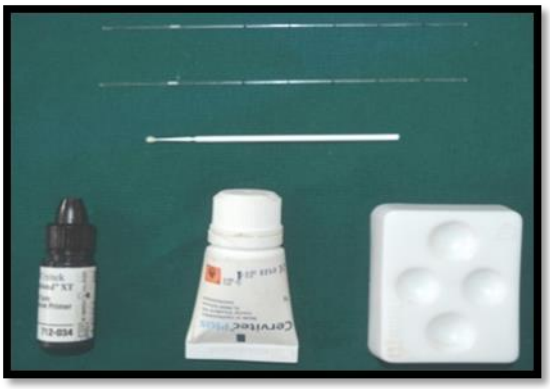

Fig 1: Accessories for mixing varnish and primer in 2:1 ratio

Brackets were debonded at the end of first month (group I), second month (group II), third month (group III), and fourth month (group IV) were tested for microbial concentration in the laboratory.

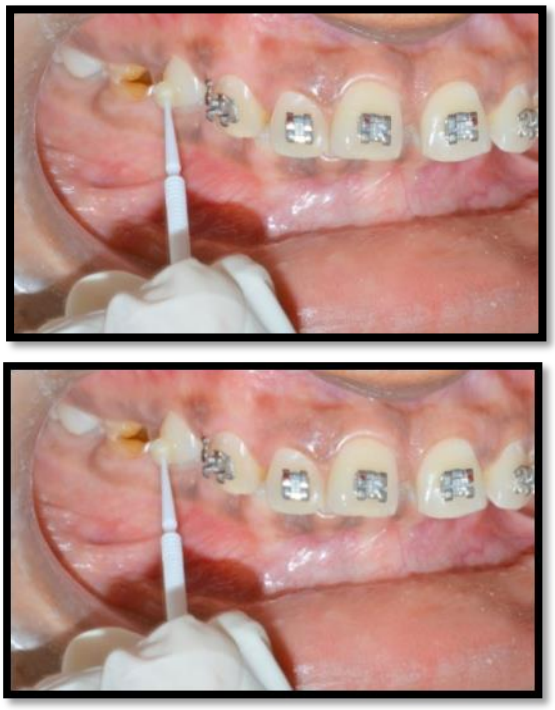

Fig 2: Varnish application (varnish and primer mixed in 2:1 ratio)

Culture procedure:

Under laminar airflow, the debonded brackets are transferred into Streptococcus selection (SS) broth and

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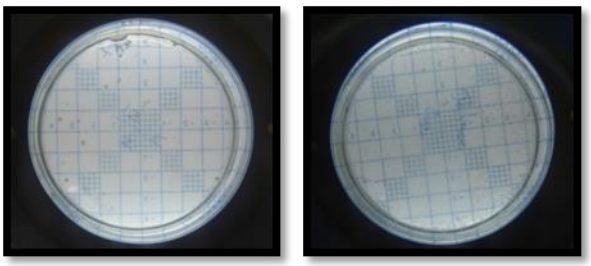
are incubated at 37ºC and 100 rpm for one day in an orbital shaking incubator.

After incubation the enriched bacterial suspension was subjected to serial dilution to 0.1ml in eppendorf tubes. 0.1ml of this diluted inoculum is transferred petri dish containing streptococcus agar using pour plate method and incubated in orbital shaking incubator at 37ºC and 100 rpm for 16-18 hours for the growth of bacterial colonies.The bacterial colonies are counted using automated colony counter machine.



Experimental group **Control group**

Calculations for 1ml is done using the formula,

Number of bacteria for 1 ml of culture =

$$\frac{\text{Colony forming units (CFU) on agar plate}}{\text{Total dilution of the tube} \times \text{Volume plated}}$$

This procedure is done for all the 40 control samples and 40 experimental samples and the results were statistically analysed.

RESULTS

The mean values of the microbial counts showed significant difference between control and experimental samples within each group. Within the controls, the microbial counts were found to be gradually increasing from a value of 5.9 ±1.3 (in 10^4) in group I to 14.9 ± 1.9 (in 10^4) in group IV. There was no substantial difference between the controls in groups III and IV. Within the experimental sample, the mean microbial counts were found to gradually increase from a value of 1.2 ± 0.6(in 10^4) in group I to 13.3 ± 2.6 (in 10^4) in group IV. All the experimental samples showed significant inhibition compared to controls in all the four groups. The highest mean inhibition percentage of 78.8% was found in group I and the least was found in group IV at 13.7 %. There was a gradual decrease in the mean inhibition percentage of microbial counts from group I to group IV (Table 1).

Group		Mean	N	Standard deviation	Standard error of mean
I	**Control**	5.9200	10	1.32732	.41974
	Experimental	1.2400	10	.60955	.19276
II	**Control**	8.9800	10	1.39666	.44166
	Experimental	2.5000	10	.94868	.30000
III	**Control**	8.4700	10	2.42581	.76711
	Experimental	5.8800	10	1.83594	.58057
IV	**Control**	14.9100	10	1.94676	.61562
	Experimental	13.3000	10	2.62467	.82999

Table 1: Mean and Standard deviation of microbial counts (Colony Forming Unit (CFU) per 1 ml in 10^4) for control and experimental samples of the four groups

Student's t test comparing the mean difference in microbial counts between the control and experimental groups showed a statistically significant difference in all the groups with a P value of < 0.005. The mean difference of 6.48 ± 1.56 (in 10^4) was found with group II which was the highest and the least was found in group IV i.e; 1.61 ± 2.17 (in 10^4) (Table 2).

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Group		Paired differences		Unpaired t test P value
		Mean	Std Deviation	
I	**Control – Experimental**	4.68000	1.19703	0.001*
II	**Control – Experimental**	6.48000	1.56971	0.001*
III	**Control – Experimental**	2.59000	1.09387	0.001*
IV	**Control – Experimental**	1.61000	2.17891	0.004*

*The mean difference is significant at the 0.05 level.

Table 2: Mean differences between control and experimental samples within the four groups.

Groups	**Group I**		**Group II**		**Group III**		**Group IV**	
	Sig	**Mean Difference**	**Sig**	**Mean Difference**	**Sig**	**Mean Difference**	**Sig**	**Mean Difference**
I	-		0.149	10.52	0.00	46.81*	0.00	66.07*
II	-		-		0.00	36.29*	0.00	55.55*
III	-		-		-		0.002	19.26*
IV	-		-		-		-	

The mean difference was significant at the 0.05 level.

Table 3: Post Hoc Tukey HSD (Honest Significant Difference) test for multiple comparisons between the four groups.

Comparison of intergroup mean microbial counts with Post hoc Tukey's HSD test at 95% confidence interval showed, a highly evident mean difference of .000 when group I was compared to group III and IV with a mean difference in the microbial count of 46.81 and 56.07 respectively. When group II was compared to group III and IV a mean difference in the microbial count of 36.29 and 55.55 respectively. The mean difference between group I and II was comparatively less with 0.149 significance level with a mean difference in the microbial count of 10.52.When group III and group IV were compared, 0.002 significance level, with a mean difference in the microbial count of 19.26 were observed.(Table 3).

Discussion

In orthodontics, composite materials are predominantly used for bonding brackets. These composites can act as a source of nutrition and agglomeration of opportunistic bacteria.[5] Hahn et al[6] reported that after polymerization, composites do not exhibit any antibacterial activity and are liable to accumulation of microorganisms around these restorative materials. Hence it would be convenient to improve existing materials to accomplish additional functions. According to Korbmacher et al[7] orthodontic bonding systems associated with sustained release antimicrobial agents are very useful because the necessity of patient compliance can be reduced. Bishara et al[8] and Damon et al[9] found that chlorhexidine varnish premixed in orthodontic primer showed adequate shear bond strength for use in orthodontic bonding. Karaman and Uysal[10] proved that when the varnish has been mixed with the primer in a 2:1 ratio, shear strength becomes clinically acceptable. Hence combination of chlorhexidine-thymol varnish pre-mixed with orthodontic primer in 2:1 ratio, was used in this study as the antimicrobial agent to test its effectiveness to prevent microbial concentration around orthodontic brackets

Chlorhexidine and thymol (Cervitec® varnish) added to orthodontic primer in 2:1 ratio, interacts and adhere to the pellicle proteins or other constituents, establishing a reserve from which chlorhexidine can be constantly delivered over time, thus reducing the amount of plaque, and the mutans streptococci (MS).

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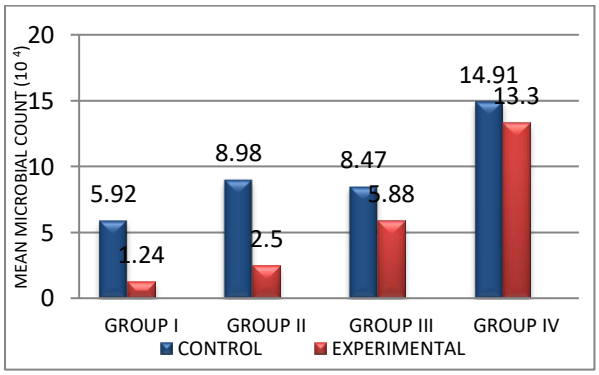
The results of the present study indicate that one-time application of chlorhexidine-thymol varnish, Cervitec® Plus with the primer during orthodontic bonding is capable of significantly reducing mutans streptococcus (MS) count at the end of first, second and third months compared to the controls. By the end of fourth month, however the effect of test varnish is comparatively declined

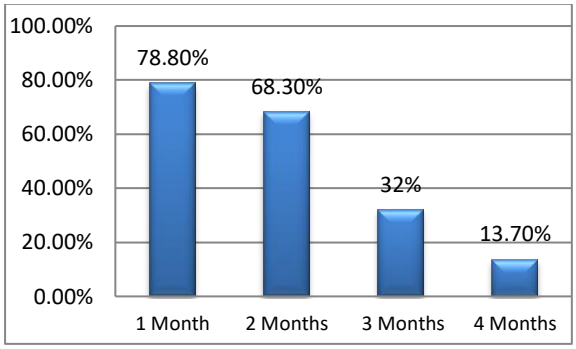
The therapeutic agents with dental biomaterials often show declining release rate since the oral fluids diffuse into the resin matrix, the antimicrobial agents mixed with the primer gets dissolved and is distributed in small concentrations. Hence this may be the reason for the decreasing trend of the antimicrobial activity in the present study with increase in time duration. As advocated by Attin et al,[4] repetition of varnish applications could achieve long-term suppression of mutans streptococci (MS) to baseline values.

The results (Graph 1) showed an increasing trend in the mean microbial counts in the controls with a highly significant increase of the count in group IV. This finding can be attributed to the coaggregation of the bacterial biofilm with increase in time as oral prophylaxis with scaling is not performed for the entire duration of the study.



Graph 1: Comparison of mean microbial counts in the control and experimental samples of the four groups.

Intragroup comparison for percentage reduction of the microbial count between the control and experimental groups (Graph 2) was found to be highly significant in group I which was 78.8%, followed by group II, III, and IV at 68.3%, 32% and 13.7% respectively. Hence a reduction in the mean inhibition percentage was found from group I to group IV, which indicates that the varnish has significant antimicrobial properties which effectively reduced the microbial concentration around the orthodontic brackets in all the four study groups.



Graph 2: Comparison of percent reduction in microbial counts between control and experimental samples within four groups.

Based on this study, it was observed that the combination of chlorhexidine varnish with an orthodontic adhesive showed effective antimicrobial activity in vivo, for the duration of 4 months. It was also noticed that there was a proportional decrease in antimicrobial activity with increase in time. The results suggested that Cervitec® plus antimicrobial varnish premixed in primer effectively reduces salivary Streptococcus mutans levels and its reapplication once in every 3 months may help to maintain sustained antibacterial effect.

From this study it is demonstrated that the sustained release devices like Chlorhexidine-thymol varnishes (Cervitec® plus) premixed in orthodontic primer is practically feasible alternative for orthodontic patients for reducing the mutans Streptococcus counts, thus preventing enamel demineralisation.

Conclusion:

Based on the findings of the present study the following interpretations can be drawn:

1. The combination of chlorhexidine-thymol antimicrobial varnish with an orthodontic adhesive showed highly evident antimicrobial activity in vivo, for the duration of four months.
2. Cervitec® plus varnish reduces salivary Streptococcus mutans levels and reapplication

every three months may help to maintain sustained antibacterial effect.

Hence use of chlorhexidine- thymol varnish premixed with primer, during bonding of brackets is a novel and feasible method to control the white spot lesions around orthodontic brackets.

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