



Comparison of Efficacy of Propolis Mouthwash With Two Other Mouthwashes against Streptococcus Mutans Concentration on Orthodontic Brackets.

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KEYWORDS

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Abstract:

Objective: To evaluate the amount of Streptococcus mutans concentration on acrylic tooth surface without bracket bonded on to it's surface and to evaluate the efficacy of Propolis mouthwash and compare it with two other mouthwashes.

Materials and Methods: A total of 50 acrylic teeth were used with 10 teeth in group A without bracket and 10 teeth with bracket in Group B,C,D and E respectively. The first part of the study compared the difference in accumulation of Streptococcus mutans between group A and B. The second part of the study compared the efficacy of Propolis mouthwash with two other mouthwashes. The samples were incubated in bottles with Brain Heart Infusion Agar inoculated with Streptococcus mutans. The samples were then treated with the respective mouthwash and the samples were estimated through Real Time PCR for the bacterial count.

Results: The mean ct values of A,B,C,D & E were 39.02,34.8,39.4,37.7 and 35.3 respectively.

Conclusion: Bonding a bracket on to the tooth surface significantly increased the concentration of Streptococcus mutans. Propolis mouthwash had statistically significant antibacterial properties against Streptococcus mutans.

INTRODUCTION:

Orthodontics is the branch in dentistry that aims in achieving an ideal occlusion, harmonious facial contours and an efficiently functioning stomatognathic system.¹ One major aspect in this branch is the prolonged treatment time that could even extend over two years. The various components of fixed orthodontic therapy makes it difficult for maintaining good oral hygiene.² With the increase in the quantity of bands and auxiliaries, there was a corresponding

quantitative increase in the microbial count.³ There is an increase in the level of Streptococcus mutans from the initial phase to the finishing phase of orthodontic therapy⁴ and after the insertion of orthodontic appliance.⁵ Streptococcus mutans are associated with the initiation of dental caries.⁶ Various changes that take place in the oral cavity of the patients during the course of treatment are the increase in the number of sites available for food accumulation, thereby increasing the Streptococcus mutans



accumulation, leading to an increased number of *S. mutans* colony-forming units (CFU) in the saliva followed by the decrease in the salivary pH.⁷ There is also an affinity of bacterial accumulation on and around orthodontic brackets.^{8, 9}

Successful orthodontic treatment is influenced by maintenance of good oral hygiene. Any compromise in maintaining proper oral hygiene can result in negative impact on orthodontic treatment results,¹⁰ leading to a 0.67 month increase in treatment time.¹¹

Normal customary maintenance of oral hygiene is achieved by mechanical brushing twice daily. Rinsing of mouth with chemical agent has proved to be an effective clinical adjunct to maintenance of good oral hygiene which reduces the plaque accumulation especially during the active phase of fixed orthodontic therapy.¹²

Chlorhexidine is considered as the “gold-standard” among the various chemotherapeutic agents that are used in the mouthwash.¹³ On the other hand it has also been associated with some adverse effects.¹⁴

Fluoride has a major role in prevention of white spot lesions which is the consequence of enamel demineralization.¹⁵ It is also shown that mouthwash containing sodium fluoride reduces the salivary levels of *Streptococcus mutans* and *lactobacilli*.^{16,17,18}

Unlike Chlorhexidine and Sodium fluoride, Propolis is a naturally occurring substance with bactericidal activity, and several studies have shown that it has potential for use in the treatment of bacterial diseases. Propolis mouthwash is found to be efficient in reducing supragingival plaque and insoluble polysaccharide formation under conditions of high plaque accumulation.¹⁹

Mouthwash containing Propolis was found to inhibit the plaque formation, thereby improving the gingival conditions.²⁰ Propolis when added to the Glass Ionomer (for banding procedures), was found to possess

antibacterial property without affecting the band strength.²¹

This in-vitro study was designed to evaluate the amount of *Streptococcus mutans* concentration on acrylic tooth surface with and without bracket bonded on to it and compare the same and evaluate the efficacy of a Propolis mouthwash on *Streptococcus mutans* concentration on orthodontic brackets and compare it with Chlorhexidine and fluoride containing mouthwash.

MATERIALS AND METHODS:

A total of 50 commercially available acrylic mandibular first premolars of size 25M (Alfadent) were included in the study and divided into five groups as follows:

Group A - 10 Teeth without bracket

Group B - 10 Teeth with bracket bonded to the buccal Surface

Group C - 10 Teeth with bracket to be treated with Chlorhexidine

Group D - 10 Teeth with bracket that is to be treated with Propolis

Group E - 10 Teeth with bracket that is to be treated with Sodium fluoride

The first part of the study evaluated and compared the concentration of *Streptococcus mutans* between teeth with and without bracket (Group A and Group B). The second part of the study was to done to compare the efficacy of Propolis mouthwash (Nature’s Goodness Propolis mouthwash) with that of Chlorhexidine (Hexidine) and Sodium fluoride (Senquel AD) (Group D, C and E).

SAMPLE PREPARATION:

Brackets used for this study was Stainless Steel brackets, .022×.028 slot size (Ormco). Brackets were bonded on to the



buccal surface using conventional orthodontic adhesive and was light cured according to the conventional procedure. The samples were then autoclaved.

STRAIN PREPARATION:

The study was carried out using MTCC (497) *Streptococcus mutans* strain, procured commercially. The bacterial cells were harvested into BHI (Brain Heart Infusion) broth to meet the turbidity Macfarland's standard 1. A total of 130 McCartney bottles were used in this study. The bottles were autoclaved and 4ml (quantity required to completely immerse the sample) of BHI broth was filled in 100 bottles. Ten microliters of 10^8 CFU (Colony Forming Units) of *Streptococcus mutans* were added to 50 bottles using micropipette and the tooth was placed into this. The bottles were segregated into the five groups and incubated at 37° C for 48 hours in an incubator.

On the day of experiment, Hexidine (4ml), Propolis (4ml) and Senquel AD (4ml) were each filled in 10 McCartney bottles respectively. The teeth with and without bracket was removed aseptically using a sterile forceps and was placed in the sterile BHI.

The tooth constituting groups C, D and E was removed aseptically using a sterile forceps and immersed into the bottle containing the respective mouthwash. The appropriate contact time was one minute and the bottle was agitated for the same. The tooth was then aseptically removed and placed in sterile BHI. The same steps were repeated for all three experimental groups.

The bottle containing the sterile BHI along with the tooth was sent to the Central

Research Facility, Sri Ramachandra University, for estimation of the bacterial count through Real Time PCR.

REAL TIME PCR ASSAY

This was done using the Fast 7900HT RT PCR equipment to study bacterial quantification by SYBR Green chemistry for relative quantification. The results were analyzed using CFQ software.

PRIMER SEQUENCE

The following primer sequence was used:

Sense (Forward) -
AGCCATGCGCAATCAACAGTT
Anti Sense (Reverse) -
CGCAACGCGAACATCTTGATCAG

STATISTICAL ANALYSIS:

The collected data was analyzed using SPSS 16.0 version. Descriptive statistics described the mean and SD. The significance of the difference between the individual groups (Teeth without bracket and Teeth with Bracket) were done using Independent t-test. Comparison between the groups (Chlorhexidine, Propolis & SenquelAD) were analysed using one way ANOVA with Tukey's Post-Hoc test. In the above statistical tools the p value .05 is considered as statistically significant.

RESULTS:

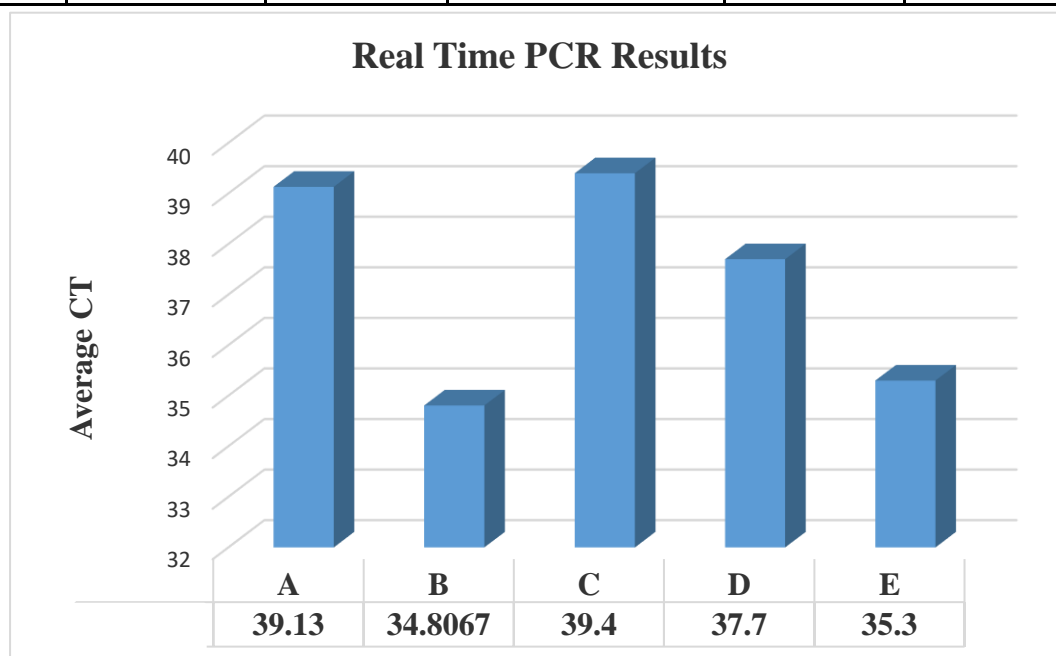
The real time PCR showed the relative quantification of *Streptococcus mutans* present in the sample by evaluating the Cycle Threshold (Ct) values. Ct values are the threshold values at which there is expression of the bacterial genome. The value is inversely proportional to the amount of bacterial genome present, which means a higher Ct value means a lower *Streptococcus mutans* count and vice versa.



The Ct values and the mean Ct values for the five groups are shown in Table 1 and Bar Diagram 1

Table 1- Ct values and the mean Ct values for the five groups

S.NO	Ct values				
	Teeth without bracket (Group A)	Teeth with Bracket (Group B)	Chlorhexidine (Group C)	Propolis (Group D)	Sodium flouride (Group E)
1	37.44	34.44	39.82	39.99	31.04
2	38.34	30.33	39.88	35.1	39.6
3	39.53	35.42	39.94	39.96	33.26
4	38.81	32.38	38.6	36.17	39.7
5	39.55	39.92	39.83	39.98	36.09
6	39.81	34.24	38.49	35	32.14
7	39.78	39.89	39.77	39.99	39.85
8	39.99	29.32	38.17	38.33	30.61
9	40.13	39.93	39.8	39.95	36.84
10	39.89	32.18	39.71	33.31	33.86
Mean	39.02	34.8	39.4	37.7	35.3



Bar Diagram 1

Comparison between the control group (Teeth with bracket) and the experimental group is shown in Table 2.



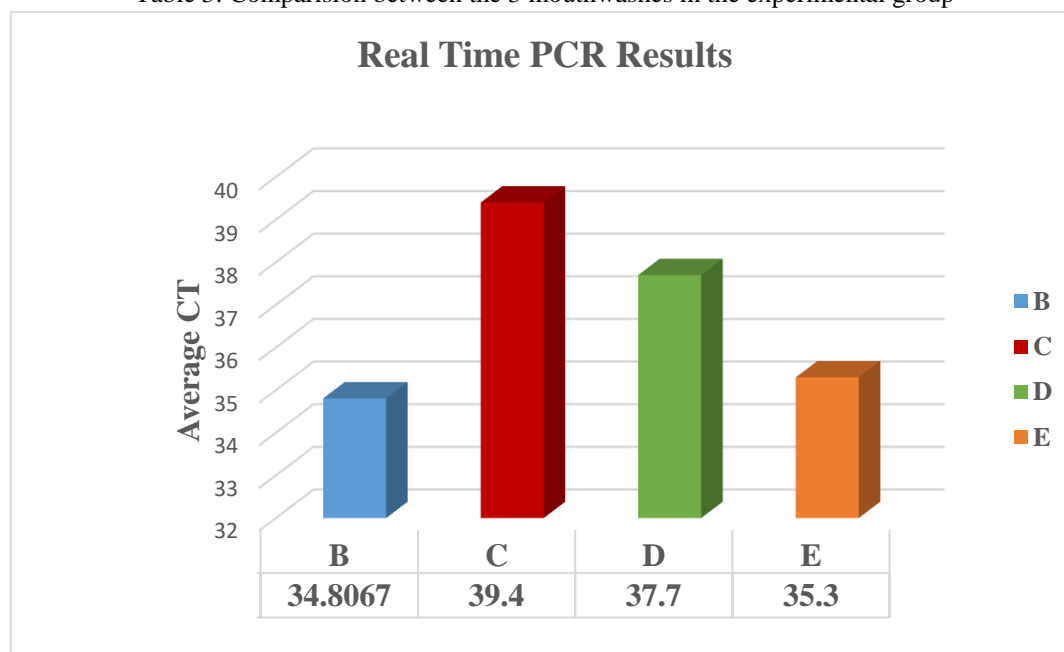
Groups	N	Mean	Std. Deviation	Significance
Teeth without bracket	10	39.02	1.36	0.000*
Teeth with bracket	10	34.8	3.89	0.000*

Table 2 : Comparison between the control group (Teeth with bracket) and the experimental group

Comparison between the 3 mouthwashes in the experimental group is shown in Table 3 and Bar Diagram 2.

GROUPS		Mean Difference	Std. Error	Significance
Teeth With Bracket	Chlorhexidine	-4.59	0.934	0.000*
	Propolis	-2.97	0.934	0.011*
	Sodium fluoride	-0.49	0.934	0.951
Chlorhexidine	Propolis	1.62	0.934	0.313
	Sodium fluoride	4.09	0.934	0.000*
Propolis	Sodium Fluoride	2.47	0.934	0.048*

Table 3: Comparison between the 3 mouthwashes in the experimental group



Bar Diagram 2



INFERENCE FROM THE RESULTS:

1. The mean Ct value for Group B (teeth with Bracket) was less than Group A (teeth without Bracket) which implies that it had greater concentration of *Streptococcus mutans*.
2. The comparison between Group A and Group B showed a statistically significant difference.
3. The mean Ct values for the experimental groups (Group C, Group D and Group E) were higher than that of the control (Group B) with Group C showing the highest value and Group E showing the lowest value.
4. Only Group C and Group D showed a statistically significant difference when compared with Group B.
5. Group E showed a statistically significant difference when compared with Group C and Group D.
6. The difference between Group C and Group D was not statistically significant.

DISCUSSION:

The true challenge for the orthodontist lies in achieving a perfectly aligned ideal occlusion with no adverse effect of the appliances used. One of the adverse effects of the fixed orthodontic appliance is the appearance of White Spot Lesions, around orthodontic brackets²² for which poor oral hygiene has been attributed as an important factor. This promotes accumulation of plaque that forms a biofilm on which adhesion of various microorganisms take place.²³

Various studies have shown a high correlation of the presence of *Streptococcus mutans* with fixed orthodontic appliance and the adhesion of *Streptococcus mutans* to the various components of fixed appliance.²⁴ Every entry of a 'poor oral hygiene' in the patient's chart, increases 0.67 month to the treatment time due factors like gingival inflammation.¹¹ Most of the patients with fixed appliance find it difficult to maintain

good oral hygiene due to the various components.²

Even though the clinician can rectify and reverse the effects of poor oral hygiene, the patient is expected to maintain good oral hygiene on a daily basis which is achieved with the help of dentifrices, mouthwash, varnish and gels which are found to significantly reduce the level of oral microflora²⁵, which seem to increase in cases of poor oral hygiene.

The use of a mouthwash in addition to professional mechanical tooth cleaning has shown to effectively reduce the count of *Streptococcus mutans*. Using a mouthwash with chemical agent has proved to be an effective adjunct for maintenance of good oral hygiene, and when used in addition to regular oral hygiene habits.¹²

Chlorhexidine has been well established as a potent antimicrobial agent against *Streptococcus mutans*. Anderson et al²⁶ showed that mouthwash containing Chlorhexidine was beneficial to a greater extent in orthodontic patients in maintaining better oral hygiene.

The effectiveness of Chlorhexidine can be attributed to its bactericidal and bacteriostatic effects and its substantivity within the oral cavity (8 hours after rinsing) however, the adverse effects of Chlorhexidine such as taste alteration, excess formation of supra gingival calculus, allergic responses and staining of teeth and soft tissues, limit their long term use.¹³

Sodium fluoride also possess antimicrobial properties but its effect is less when compared to that of Chlorhexidine.^{16 17} Propolis is a naturally-occurring substance. Propolis chiefly consists of wax and plant extracts. Propolis is used by the bees to seal the hives and it plays a major protective role against invasion and infection.²⁰

Flavones, flavanones and flavanols are the major components of Propolis.²⁰ Components present in propolis like apigenin



was found to have proven activity against glucosyltransferases and tt-farnesol.²⁷

Various studies have shown the antibacterial properties of Propolis against *Streptococcus mutans* when used in the form of dentifrice and mouthwash.^{19,28} Propolis was also found to effectively reduce the amount of plaque accumulation and polysaccharide formation.¹⁹

The cytotoxic level of Propolis on human gingival fibroblast were comparatively less when compared with chlorhexidine since Propolis was found to have mucoprotective properties.²⁹

This study compared the difference between the adhesion of *Streptococcus mutans* to acrylic tooth surface with and without bracket bonded on to its surface. The adhesion of *Streptococcus mutans* is greatly influenced by surface free energy and surface roughness.³⁰ Thus selection of acrylic teeth was done in this study to standardize the surface free energy and surface roughness. Mandibular first premolars were only selected for the purpose of standardizing the size.

The study also compared the efficacy of Propolis mouthwash with Chlorhexidine and Senquel AD against *Streptococcus mutans* accumulated on orthodontic brackets.

The study showed that bonding of the bracket on to the tooth surface significantly increases the *Streptococcus mutans* accumulation which was similar to the finding of other studies.^{7,31}

Comparing the antibacterial properties of the three mouthwashes, Chlorhexidine had the maximum efficacy against *Streptococcus mutans* followed by Propolis. Senquel AD had the least antibacterial property. Regarding the antibacterial properties of Propolis, the finding was similar to other studies.^{20,29,32,33}

The finding of this study showed that there was not a statistically significant difference between the antibacterial properties of Propolis and Chlorhexidine.

CONCLUSION:

1. Bonding a bracket on to the tooth surface significantly increased the concentration of *Streptococcus mutans*.
2. Chlorhexidine and Propolis had significant antibacterial effect against *Streptococcus mutans* with Chlorhexidine having the highest antibacterial activity but with no statistically significant difference.
3. Even though Sodium fluoride possessed antibacterial properties, the effect was not statistically significant when compared with the control.
4. Further in-vivo research has to be done to confirm the antibacterial efficacy of Propolis.

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