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Comparison of Antimicrobial Efficacy of Custom-Made Orthodontic Adhesives- A Double Blinded In-Vivo Study

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Abstract

Introduction: This double blinded study was designed to investigate the antimicrobial efficacy of three custom modified antimicrobial orthodontic adhesive against the bacterial load of Streptococcus mutans around orthodontic brackets over 28-days period. Materials and Methods: A total of 56 teeth from 14 male prospective orthodontic patients in the age group of 15-30 years were drawn for the study. The samples were divided into four groups, Group 1 contains Transbond XT primer, Group 2 contains Transbond XT modified Cetylpyridinium Chloride, Group 3 contains Transbond XT modified Triclosan and Group 4 contains Transbond XT modified Sodium Fluoride. Transbond XT primer was modified by addition of 2.5% Cetylpyridinium chloride, 0.3% Triclosan and 2% Sodium fluoride using a magnetic stirrer. Buccal surface of maxillary and mandibular lateral incisors was etched with 37% phosphoric acid gel and modified and control primers were applied over the etched surface and light cured. Metal brackets were bonded directly using Transbond XT composite and modules were engaged over it. On day 1,7 and 28 the modules were collected from the brackets for the bacterial count. The bacteriological study was conducted by Dilution Plating Method at baseline, 1st day, 7th day and 28th day. Results: On intragroup comparison all the antimicrobial agents showed maximum activity at day 7. Upon intergroup comparison at 1st Day, 7th Day and 21st Day, maximum antimicrobial activity was seen in Sodium Fluoride, Triclosan and Cetylpyridinium Chloride group respectively. Conclusion: The performance when judged over the total study period of 28 days indicates all modified primer showed effective antimicrobial activity and among them Triclosan had the better antimicrobial action in primer against Streptococcus mutans.

Keywords: Antimicrobial, Cetylpyridinium Chloride, Triclosan, Sodium Fluoride.

Introduction

Delivering of orthodontic treatment outcomes are often compromised by many host variables; the primary amongst them being noncompliance to oral hygiene instructions resulting in high accumulation of biofilm and plaque. The biofilm naturally found around teeth is a microcosm of a variety of microorganisms embedded and adherent on the surface. Orthodontic patients have higher amount of biofilm and Memon et al [1] documented that brackets, arch wires and other orthodontic gadgets are main focal points for accumulation of plaque and also act as obstacle in plaque control and enhance gingivitis. Accompanied by poor or inadequate brushing, the oral hygiene deteriorates with the drop in pH in the oral cavity. Altered remineralization due to high acidic pH by incriminating organism like streptococcus mutans, lactobacilli create demineralization resulting in incipient caries known as white spot lesions(WSL). According to Tufekci et al [2], WSLs can become noticeable around the brackets within 1 month of bracket placement. Gorelick et al [3], using the visual examination technique, reported that 50% of patients had one or more WSLs at the end of orthodontic treatment.

Chlorhexidine, Cetylpyridinium Chloride, Triclosan, Sodium Fluoride has shown effective antibacterial activity and has been used widely in different oral hygiene aids, but use of these oral hygiene aids largely depends on patient compliance during orthodontic treatment. Modification of orthodontic bonding materials with inclusion of various antimicrobial agents in orthodontic adhesives with a magnetic stirrer has been used for prevention of white spot lesions has been demonstrated by Chung et al [4] by using Chlorhexidine and Musallam et al [5], using Cetylpyridinium Chloride to a commercial photo activated orthodontic adhesive. Garza et al [6] showed that Triclosan and Cetylpyridinium Chloride can be incorporated into bis-acryl resin to inhibit the growth of plaque bacteria. Similarly, Badawi et al [7] found that use of Fluoride containing orthodontic materials like GIC and composite resins (both in vitro and in vivo) reduced decalcification around orthodontic brackets. This process of mixing adhesive is technique sensitive as a homogenous mix of adhesive is often a challenge. So in this study, it was decided to modify primer to assess if custom modified primers with antimicrobial agents could reduce the bacterial load of Streptococcus mutans around orthodontic brackets over 28 days.

Materials and Methods

A total of 56 teeth from 14 male prospective orthodontic patients in the age group of 15-30 years were drawn for the study. The teeth included were lateral incisor from maxillary and mandibular arch of each patient. This study was approved by Institutional Committee and a written informed consent was obtained from the participating patients.

Following inclusion criteria were used for patient selection:

- Good general and periodontal health.
- Patients with similar socioeconomic strata and common food habits
- Patients free of oral/parenteral antibiotics during study period
- Absence of any systemic condition that could affect periodontal status and microbial count.

Following Exclusion criteria were used for patient selection:

- Medically compromised patients.
- Patients who are mentally or physically challenged.
- Presence of decalcification of teeth.
- Presence of anterior composites.

- Presence of crowding.

Participating patients and the investigator were blinded to the intervention allocated to each group by the supervisor of the research work. The subjects were given oral hygiene instructions and requested to refrain from using any other oral hygiene products like mouthwash. They were instructed to follow standard oral hygiene regime which included brushing twice a day with toothpaste and advised to rinse with water thoroughly after every meal. Two days prior to bonding, buccal surface prophylaxis was performed using a rubber cup and pumice and water.

A) Procedure For Fabrication Of Custom Made Primer

2.5% Cetylpyridinium Chloride, 0.3% Triclosan and 2% Sodium Fluoride was weighed using an electronic weighing machine and incorporated into 1ml of Transbond XT primer in a mixing flask and was stirred for 12hours using Magnetic stirrer at room temperature under red light in a dark room to obtain uniform mixtures. Thus the samples were divided into 4 groups.

Groups	Sample	Code For Blinding	TestVariables Of Primers
Group I	N=14	Op1	Transbond Xt Primer (Control)
Group II	N=14	Op2	Transbond Xt Incorporated Cpc
Group III	N=14	Op3	Transbond Xt Incorporated Triclosan
Group IV	N=14	Op4	Transbond Xt Incorporated Naf

Table No. 1: Sample Segregation

B) Bonding Procedure

Buccal surface of maxillary and mandibular lateral incisors was acid etched with 37% phosphoric acid gel for 30 seconds followed by rinse for 30 seconds and then dry with oil free compressed air for 20 seconds. Modified and control primers were applied over the etched surface of lateral incisors and light cured for 20 seconds. Metal brackets were bonded directly using Transbond XT composite. Flash around the bracket was removed using an explorer tip followed by light cured for 20 seconds. After placing the bracket, modules were used to engage over it.

C) Plaque Collection Method

Patients were requested to refrain from eating or drinking 1 hour prior to sample collection. Plaque samples were collected at baseline, 1 day, 7 days and 28 days respectively and were placed in sterilized vials having saline in it. Plaque were collected from the facial surface of the maxillary and mandibular lateral incisor at day 0 before bracket placement by using a cotton swab. On day 1, the modules were collected from the brackets for the bacterial count. A new module was placed again and bacterial count was done after 7 days and 28 days. These modules were transported to lab using 5ml sterilized vials with 1ml saline in it.

D) Lab Procedures

The bacteriological study was conducted by Dilution Plating Method. Mitis Sanguis Agar was mixed in 1 L of distilled water and sterilized at 121℃ for 20 minutes in an Autoclave. Serial 10 fold dilutions were made for each sample till 10^{-3} and 0.1ml of sample was then plated on Mitis Sanguis Agar plates with 0.1% bacitracin which is a selective culture media for S. mutans. The number of colonies were counted with a Digital Colony Counter and expressed as Colony forming units per ml (CFU/ml).

Results

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Upper Right	14	123.50	15.815	4.227	114.37	132.63	99	159
Upper Left	14	110.71	10.593	2.831	104.60	116.83	95	128
Lower Left	14	113.43	11.230	3.001	106.94	119.91	95	130
Lower Right	14	124.93	12.474	3.334	117.73	132.13	105	146

Table No. 2: - Distribution of Mean and S.D. bacteria count (CFU/ml) at 0th Day of four groups

ANOVA					
	Sum of Squares	Df	Mean Square	F	P value
Between Groups	2130.143	3	710.048	4.410	.008**
Within Groups	8372.714	52	161.014		
Total	10502.857	55			

Table No. 3: - Comparison of Mean bacteria count (CFU/ml) at 0th Day of four groups by one way of ANOVA

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1C	14	173.43	19.194	5.130	162.35	184.51	145	210
2CP	14	146.93	22.886	6.117	133.71	160.14	121	195
3T	14	158.21	23.146	6.186	144.85	171.58	131	208
4F	14	149.71	14.636	3.912	141.26	158.17	130	180

Table No. 4: - Distribution of Mean and S.D. bacteria count (CFU/ml) at 01th Day of four groups

ANOVA					
	Sum of Squares	df	Mean Square	F	P value
Between Groups	5962.143	3	1987.381	4.841	.005*

Within Groups	21347.571	52	410.530		
Total	27309.714	55			

Table No.5: - Comparison of Mean bacteria count (CFU/ml) at 01th Day of four groups by one way of ANOVA

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1C	14	-42.7367	25.65811	6.85742	-57.5513	-27.9222	-95.96	-4.32
2CP	14	-32.9065	17.28611	4.61991	-42.8872	-22.9258	-64.04	-6.67
3T	14	-41.0034	26.31333	7.03253	-56.1963	-25.8105	-89.09	-9.76
4F	14	-20.4279	11.56241	3.09018	-27.1038	-13.7520	-35.78	6.16

Table No. 6: - Distribution of Mean and S.D. of % change between Day 0 and Day 1 bacteria count (CFU/ml) of four groups

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1C	14	169.07	18.620	4.976	158.32	179.82	147	205
2CP	14	115.07	20.402	5.453	103.29	126.85	85	154
3T	14	107.14	11.016	2.944	100.78	113.50	92	126
4F	14	118.21	13.740	3.672	110.28	126.15	103	150

Table No. 7: - Distribution of Mean and S.D. bacteria count (CFU/ml) at 7th Day of four groups

ANOVA					
	Sum of Squares	df	Mean Square	F	P value
Between Groups	33365.196	3	11121.732	41.458	<.001**
Within Groups	13949.929	52	268.268		
Total	47315.125	55			

Table No. 8: - Comparison of Mean bacteria count (CFU/ml) at 7th Day of four groups by one way of ANOVA

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1C	14	1.6201	14.04391	3.75339	-6.4886	9.7288	-26.21	24.23
2CP	14	20.5284	15.36728	4.10708	11.6556	29.4012	-9.22	48.66

3T	14	31.0612	11.31193	3.02324	24.5299	37.5925	12.21	55.29
4F	14	20.8609	7.31099	1.95394	16.6396	25.0821	5.63	33.12

Table 9: Distribution of Mean and S.D. of % change between Day 1 and Day 7 bacteria count (CFU/ml) of four groups.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1C	14	177.00	18.982	5.073	166.04	187.96	148	205
2CP	14	120.86	15.674	4.189	111.81	129.91	103	155
3T	14	120.00	10.243	2.738	114.09	125.91	102	137
4F	14	136.57	9.788	2.616	130.92	142.22	120	158

Table No. 10: - Distribution of Mean and S.D. bacteria count (CFU/ml) at 28th Day of four groups

ANOVA					
	Sum of Squares	df	Mean Square	F	P value
Between Groups	29952.214	3	9984.071	49.506	<0.001**
Within Groups	10487.143	52	201.676		
Total	40439.357	55			

Table No.11: - Comparison of Mean bacteria count (CFU/ml) at 28th Day of four groups by one way of ANOVA

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1C	14	-4.0079	9.93185	2.65440	-9.7424	1.7266	-21.99	11.39
2CP	14	-4.7872	11.93873	3.19076	-11.6804	2.1060	-20.93	28.33
3T	14	-10.4948	7.98189	2.13325	-15.1034	-5.8862	-27.56	-.86
4F	14	-13.0019	11.82272	3.15975	-19.8281	-6.1757	-34.81	8.33

Table 12: Distribution of Mean and S.D. of % change between Day 7 and Day 28 bacteria count (CFU/ml) of four groups

%Change Day0&Day28								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1C	14	-45.3426	22.57362	6.03305	-58.3762	-32.3090	-72.27	.63
2CP	14	-10.0439	17.62266	4.70985	-20.2190	.1311	-44.79	14.17
3T	14	-6.4500	10.73031	2.86780	-12.6455	-.2546	-19.19	17.07
4F	14	-10.2749	12.96337	3.46461	-17.7597	-2.7900	-33.03	11.11

Table No. 13: - Distribution of Mean and S.D. of % change between Day 0 and Day 28 bacteria count (CFU/ml) of four groups.

At baseline prior to application of any intervention the lowest count was seen in the upper left side followed by lower left side and upper right side, while the highest count was in the lower right side of the mouth. Maxilla showed the least microbial count when compared with the mandible. At day 1, there was an increase in microbial count seen in all 4 groups and minimum % change (increment) of bacteria at 1st day was seen in Sodium Fluoride group. There was a statistically significant mean difference of bacteria count (CFU/ml) at 01th Day between all four groups $p \leq 0.05$. When compared with day 1, all the group at day 7 shows significant reduction in microbial count. Triclosan and Sodium Fluoride at Day 7 showed microbial count that has gone below the baseline value, which means that they are highly effective in controlling growth of microorganisms. The Maximum % change (reduction) at 7th day in Triclosan group. By one-way ANOVA there was a high significant mean difference of bacteria count (CFU/ml) at 7th Day between four groups, $p < 0.01$. On multiple comparison by post hoc Tukey's HSD test, the mean difference of bacteria counts (CFU/ml) at 7th Day between groups Control and Cetylpyridinium Chloride, Control and Triclosan, Control and Sodium Fluoride are highly significant, $p<0.01$. At day 28, there was an increase in microbial count for all group when compared with Day 7. The Minimum % change (increment) of bacteria at 28th day in Cetylpyridinium Chloride group. Bacteria reduction is Maximum in Triclosan group on 7th day and increment from 7th -28th day is not significant so Triclosan is better than all other groups $p > 0.05$. On Overall (0-28 days) the Minimum % increment in bacteria count was found in Triclosan group and there was a significant difference in mean % change between Day 0 and day 28 between four groups $p<0.01$.

Discussion

Several orthodontic adhesives containing antimicrobial agent are commercially available but according to Ozel [8] they exhibit limited or no intrinsic activity against microorganisms. Study by Melo et al [9] has shown the addition of antimicrobial agent like Fluoride and Chlorhexidine into orthodontic adhesives by physical blending. However, these agents had some disadvantages, such as short-term release of antimicrobial agents and decreased mechanical properties.

Study by Chung et al [4] on addition of Chlorhexidine into orthodontic adhesive found that adhesive-enamel interface deteriorates due to cyclic fatigue exerted during

mastication, which induces microgaps at the interface. Microgaps can harbor biofilms, and their acid production can cause demineralization at the bracket margin. Therefore, it is desirable that primers contain antimicrobial property because the invading bacteria in marginal gaps directly contact primer rather than the enamel surface. Study by Ozel et al [8] found that incorporation of Cetylpyridinium Chloride in Transbond XT™ adhesive primer has been shown to confer significant antibacterial activity, sustained release and superior shear bond strength with no significant change in the mode of bracket failure under shear stress.

In this current research, at Day 0, prior to application of any intervention the mean values of streptococcus mutans count varied in all 4 segments. The lowest count was seen in the upper left side. The right side of the patient teeth had more microbial count than the left side. This might be because most of our patients uses right hand to brush their teeth and might have got more brushing strokes on the left side of mouth.

On Day 1, after the application of modified primer, brackets and modules, the microbial count got increased in all 4 groups when compared with Day 0. Overall this increase in microbial count may be due to the more adhesion of bacteria over elastomeric modules or because of improper brushing technique. Among them, the lowest mean difference and % change in microbial count was seen with Fluoride group followed by Cetylpyridinium Chloride and Triclosan group. while the highest was seen with the Control group. This result was similar with the study done by Ozturk [10], Kassis [11] on Fluoride, where they found that the release was high on the first day, fell rapidly over the next day, then gradually decreased to a nearly constant level by the end of the third day.

At Day 7, when compared with day 1, all group showed a significant reduction in microbial count. The highest antimicrobial activity on account of mean difference and % change was seen with Triclosan followed by Sodium fluoride and Cetylpyridinium Chloride group, while Control group did not show any reduction in microbial count. When compared with Day 0, Triclosan and Sodium Fluoride at Day 7 showed microbial count that has gone below the baseline value, which means that they are highly effective in controlling growth of microorganisms. This was in contrast to the study done by Degrazia [12] on Triclosan, where they found that the release of antimicrobial agent was high on 24hr, which fell rapidly over 48hrs and 72hrs for all experimental groups.

At day 28, there was an increase in microbial count for all group when compared with Day 7. Antimicrobial activity of all modified primer has become consistently reduced at Day 28 when compared with Day 7. These are in accordance to study done by Musallam et al [5] on CPC in which as time of aging increased, there seemed to be a slight decrease in the zones of bacterial inhibition. At Day 14, the antimicrobial activity of the modified discs decreased when compared with day 1 but was similar to the end of the 196-day storage.

The results of the current research were similar to the study done by Singh et al [13] on addition of Chlorhexidine in Transbond XT primer and found that an initial surge was reported in the first week, with large inhibition zones at the time of first sampling, and, later, these decreased in size continuously on the second sampling at the 12th day and at the third sampling on the 18th day, showing marked reduction of the Chlorhexidine activity over a period of 2 weeks. However, after this time, the result obtained at the next sampling periods at the 24th day until the completion of the last sampling, which was completed on the 60th day, was more or less constant. The disadvantage found with adding CHX into primer was that CHX is partly water soluble and elution of CHX may decrease its long-

term antimicrobial activity, because of limited quantities in the primer.

According to Chung et al [4] an appropriate concentration of antimicrobial agents in the primer is important because insufficient amounts limit antimicrobial activity and excess amounts deteriorate the mechanical properties of materials. According to Degrazia [12], greater the concentration of antimicrobial agent in adhesives the lower is the CFU counts. In the current study a concentration of 2.5% CPC, 0.3% Triclosan and 2% Sodium Fluoride was used which showed maximum effect at day 7 and effectiveness got reduced as aging advanced. So further studies on different concentration is required which will determine the longevity of antimicrobial agents.

This study has clearly shown that Cetylpyridinium Chloride, Triclosan and Sodium Fluoride when mixed with the primer of the bonding adhesive provides antimicrobial activity, which is derived at the site of plaque accumulation and is sustained over a period of time. This has a possibility to decrease the demineralization zone present at these sites at the time of debonding. Since 28 days may be not enough to analyze long-term antimicrobial effects of primers because the orthodontic treatment will last for about 18-24 months. Further long-term in vivo studies are needed to investigate the physical and antimicrobial potentials of orthodontic primers and test validity of these modified primer to sustain clinical masticatory and other stresses during the total period of orthodontic treatment.

Conclusion

- The three test variables- Triclosan, Cetylpyridinium Chloride and Sodium Fluoride performed almost without any intergroup difference in controlling the bacterial accumulation in and around the brackets.
- The performance when judged over the total study period of 28 days indicates Triclosan as a better antimicrobial agent in primer against Streptococcus mutans.
- The performance of Triclosan, Cetylpyridinium Chloride and Sodium Fluoride reduced over the time period of the study in all probability due to elution in the oral cavity.
- Further long duration, multi drug study with varying dilution would provide deeper insights into understanding effect of microbial agents introduced into bonding adhesives.
- The validity of the custom modified primers to reduce or control white spot lesion and demineralization need to be weighed against clinical benefits of bond strength and other factors to check total performance under occlusal and masticatory stresses over the complete duration of orthodontic treatment.

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