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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL AND PHYSICAL PROPERTIES OF ORTHODONTIC COMPOSITE RESIN MODIFIED BY ADDITION OF ANTIMICROBIAL AGENTS – AN IN-VITRO STUDY

***Dr. Tarush Thakur, Dr. Rohit Gupta, Dr. Aprajita Dogra and Dr. Shipra Mahajan**

MDS, Department of Orthodontics and Dentofacial Orthopaedics, Himachal Dental College, Sundernagar, Himachal Pradesh

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ABSTRACT

Introduction: Patients who undergo orthodontic therapy also have changes in their oral ecologic, such as a low-pH environment, increased retentive sites for Streptococcus mutans, preventive strategies should be independent of patient cooperation, and the drug should be released over a prolonged period of time. Hence incorporation of antibacterial agents in orthodontic adhesivess provide desired results. **Aims and objectives**: To assess the antimicrobial properties of orthodontic composite resin when combined with various antimicrobial agents and their effect on the mechanical properties of the composite material along with the evaluation of the release of anti-microbial agents from the modified composite. **Material and Methods:** 100 extracted premolars divided into 4 groups (25each)and each group was bonded with composite adhesive mixed with respective anti-microbial agent Benzalkonium Chloride 0.1% (w/w) ,Chlorhexidine 0.2% (w/w) and Triclosan :0.3% (w/w)The statistical analysis was done using IBM SPSS (Statistical package for social sciences) software (Version 21.0). **Conclusion:** Benzalkonium chloride modified adhesive showed better dependability and clinical reliability as compared to Triclosan modified orthodontic adhesive group and Chlorhexidine modified orthodontic adhesive group.

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INTRODUCTION

The significant challenge in fixed orthodontic appliance therapy is to maintain good oral hygiene to avoid or minimize decalcification of enamel during treatment.[1] Once the orthodontic fixed appliance is bonded into the oral cavity, a rapid shift in the bacterial flora of plaque occurs. Numerous appliance components like brackets, archwires and bands are a focus for plaque accumulation and an obstruction to plaque removal, thereby promoting gingivitisand predisposing to the white spot lesions. Plaque also harbors cariogenic bacteria potentially capable of hard tissue damage, especially at the bracket margins.[3,4] The incipient lesions appear as early as two weeks after plaque accumulation in buccolingual areas of the teeth.[5] Higher levels of acidogenic bacteria are present in the plaque, most notably *Streptococcus mutans* and *Lactobacilli*[7] which are capable of decreasing the pH of plaque in orthodontic patients to a greater extent than in non-orthodontic patients.[8] Therefore, the progression of caries is faster in patients undergoing orthodontic therapy. White spot lesion is one of the most common problems faced after the start of orthodontic treatment.

****Corresponding author:*** **Dr. Tarush Thakur,**
MDS, Department of Orthodontics and Dentofacial Orthopaedics, Himachal Dental College, Sundernagar, Himachal Pradesh.

They become noticeable around the brackets within 1 month of bracket placement, although the formation of regular caries usually takes at least 6 months.[9] These lesions are commonly seen on the buccal surfaces of teeth around the brackets, especially in the gingival region.[3,10,11] The lesions are not distinct types of carious lesions; rather they are the result of enamel demineralization as a stage of the carious process occurring around orthodontic fixed appliances.Frequent exposure to fermentable carbohydrates leads to more intense and frequent acidification of the biofilm. This in turn leads to the adaptive and selective modification of the biofilm to favour more acidogenic and aciduric strains of microflora. This negative modification of the biofilm leads to a shift in the demineralization/remineralization cycle toward a net mineral loss.[24] While fluoride varnishes, fluoride mouth rinses, and oral hygiene instructions have been employed to inhibit White spot lesions, they rely heavily on patient compliance and provide only intermittent protection against decalcification. Bonding agents that release fluoride show rapidly decreasing levels after the first 24 hours.[25,26]For an effective remineralization process, it is necessary to control the bacterial biofilm around the brackets and maintain a constant presence of fluoride in the oral cavity.[27] Antimicrobials have also been suggested as an adjunct for those patients with a higher caries risk. While repeated at-home applications of antimicrobials by the patient may reduce the patient's caries risk,[30] patient compliance in

using these materials is the critical factor. As a result, the addition of antimicrobials to the adhesive system would eliminate the need for patient cooperation and thus would have an obvious advantage. Ever since the introduction of enamel etching byBuonocore[31] (1955) and composite resins to the field by Newman[32] (1965), the quality and ease of handling of bonding materials have improved many folds, but little attention was given to enhance their antimicrobial properties. Recently, researchers[34,35] modified filling materials by adding antimicrobial agents such as Chlorhexidine and quaternary ammonium compounds to composite resins and acrylic resins. They found that these agents, added in minute quantity, could impart an antibacterial trait to dental materials without significantly affecting their physical properties. In the present study, light cured composite bonding agent was modified by addition of benzalkonium chloride (BAC), chlorhexidine and triclosan as anti- microbial agents and their efficacy was evaluated. This study also evaluated the leaching of these antimicrobial agents from the composite resin at definite time intervals and the effect of ageing on shear bond strength of these modified composite resins. Hence, the aims and objectives of the present in vitro study were to assess the antimicrobial properties of orthodontic composite resin when combined with various antimicrobial agents and their effect on the mechanical properties of the composite material along with the evaluation of the release of anti-microbial agents from the modified composite resin.

MATERIALS AND METHODS

The present in vitro study was conducted at the Department of Orthodontics and Dentofacial Orthopaedics and Department of microbiology on 100 non-carious, unrestored freshly extracted premolar teeth were collected with the following inclusion and exclusion criteria:

Inclusion criteria of teeth selection

- Extractions done for orthodontic purposes
- Non-carious, unrestored teeth.
- Teeth not subjected to any pre-treatment chemical agents.
- Teeth without developmental defects on the enamel surfaces.

Exclusion criteria of teeth selection

- Extensive wear.
- Cracked enamel or fractured cusps.
- Hypoplastic and irregular structure.

In the first part of the study, minimum inhibitory concentration (MIC) of the antimicrobial agents were found out by the macrobroth dilution method. The antimicrobial agents were procured in the powder form and were used as such. The concentrations of antimicrobial agents used were as follows:

- Benzalkonium Chloride (Somu Organo-Chem, Bangalore): 0.1% (w/w)
- Chlorhexidine (Kumar organic products limited, Bangalore) :0.2% (w/w)
- Triclosan (Kumar organic products limited, Bangalore): 0.3% (w/w)

A premolar with bracket (0.022" Slot MBT, Gemini Series 3M Unitek) bonded using orthodontic adhesive(Transbond XT (3M Unitek) along with Transbond XT Primer) mixed with antimicrobial agent was used to find out the zone of inhibition on a culture of *S. mutans*.

These were divided into 4 groups

Group I: 25 teeth bonded with Transbond adhesive mixed with Benzalkonium chloride.

Group II: 25 teeth bonded with Transbond adhesive mixed with Chlorhexidine.

Group III: 25 teeth bonded with Transbond adhesive mixed with Triclosan.

Group IV: Control (25 teeth bonded with Transbond adhesive without any addition).

Blood Agar plate was used, on which lawn culture of *S mutans* was prepared by swabbing. A premolar was then placed at the centre of the agar plate which was then incubated for 24 hrs at 37°C (figure..). Another premolar with bracket bonded using orthodontic adhesive mixed with antimicrobial agent was placed in the 1ml tryptic soy broth and checked for bacterial growth after incubating at 37°C for 24 hrs(figure…) This depicted the leaching of antibiotic from the modified adhesive and its bactericidal action in liquid nutrient media. Four sets of 25 premolars with bracket bonded using orthodontic adhesive mixed with antimicrobial agent, each placed under artificial saliva were used to find out the time dependent release of antimicrobial agents from the composite adhesives, which was monitored spectrophotometrically (Systronics Double Beam UV-VIS Spectrophotometer: 2202).Reading were taken at 24hrs and at days 5, 10, 15, 20and25 (figure….)

The study required the premolars to be divided into two sets. One set of teeth was tested twenty-four hours after bonding; second set of teeth was tested after twenty-five days of storage in distilled water and were randomly allocated to four groups (figure…). Shear bond strength was tested with a universal testing machine (Llyod Instruments UTM LR 50K) with a crosshead speed of 0.5 mm per minute (figure….).Shear bond strengths after 24 hours of bonding and after 25 days of bonding were compared using unpaired t Tests. The bond failure rate was also determined using Weibull analysis. The leaching of the antimicrobial agent after immersion in artificial saliva was determined with spectrophotometric analysis.

RESULTS

The present study was carried out on 100 extracted premolars divided into 4 groups of 10 each to be tested at 24hrs after bracket bonding and 4 groups of 15 each to be tested after 25 days of bracket bonding respectively. The statistical analysis was done using IBM SPSS (Statistical package for social sciences) software (Version 21.0). The descriptive statistics for shear bond strength including the mean, standard deviation, minimum and maximum values for each of four groups were calculated (Table I). Shear bond strengths after 24 hours of bonding and after 25 days of bonding were compared using unpaired t Tests (Table II, Graph I). The bond failure rate was also determined using Weibull analysis (Table III).

Table I : descriptive analysis of bond strengths at 24 hrs and after 25 days of immersion in artificial saliva

Time		Groups	Sample size	Mean (Mpa)	Standard Deviation	Standard Error	Min. Value (Mpa)	Max. Value (Mpa)
24 Hours	I	Control	10	12.605	3.16	0.99	5.7	16.1
	II	Benzalkonium chloride	10	11.295	1.94	0.61	9.62	15.75
	III	Triclosan	10	10.632	1.77	0.56	8.04	14.05
	IV	Chlorhexidine	10	11.311	2.04	0.64	9.32	14.79
25 Days	I	Control	15	11.39	1.96	0.50	9	14.89
	II	Benzalkonium chloride	15	12.65	2.11	0.54	9.1	15.54
	III	Triclosan	15	11.09	1.39	0.35	9.5	14.54
	IV	Chlorhexidine	15	11.51	1.93	0.49	8.63	14.74

Table II : Comparison Of Bond Strengths At 24 Hours After Bracket Bonding And After 25 Days Of Bracket Bonding Using T Test.

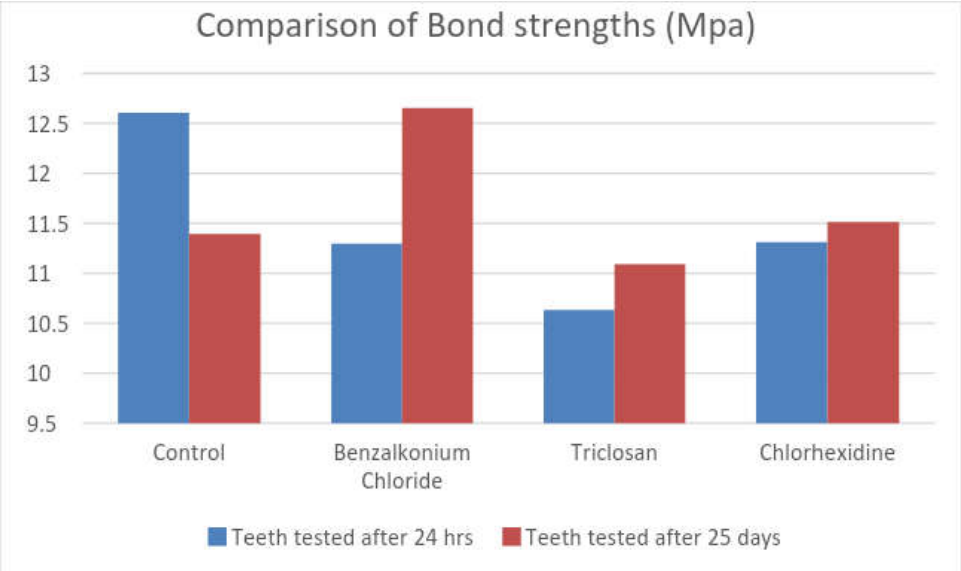
Group	Mean Bond Strength at 24 hrs	Mean Bond Strength after 25 days	P Value
Control	12.605	11.39	0.17
Benzalkonium Chloride	11.295	12.65	0.11
Triclosan	10.632	11.09	0.20
Chlorhexidine	11.311	11.51	0.46

Table III : Comparison of all groups with weibull analysis to determine clinical reliability of the material

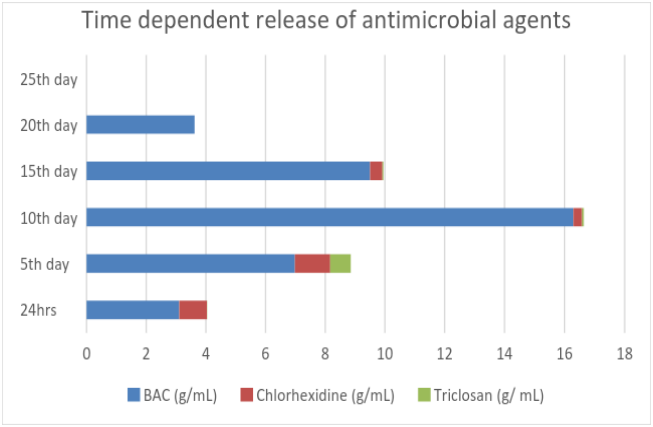
Time		Group	Mean Bond Strength	Weibull Modulus	Normalizing Parameter	R^2
24 hrs	I	Control	12.605	3.34	14.1	0.88
	II	Benzalkonium Chloride	11.295	6.03	12.17	0.74
	III	Triclosan	10.632	6.54	11.37	0.91
	IV	Chlorhexidine	11.311	5.77	12.21	0.81
25 days	I	Control	11.39	11.52	10.65	0.89
	II	Benzalkonium Chloride	12.65	7.12	12.36	0.94
	III	Triclosan	11.09	15.67	10.67	0.88
	IV	Chlorhexidine	11.51	9.02	10.97	0.94

Table IV : Time Dependent Release Of Antimicrobial Agents.

Group No.		Time					
	Antimicrobial agents	24hrs	5th day	10th day	15th day	20th day	25th day
II	Benzalkonium chloride(µg/mL)	3.103	6.982	16.29	9.5	3.62	0
III	Triclosan (µg/ mL)	0.01	0.69	0.057	0.031	0	0
IV	Chlorhexidine (µg/mL)	0.928	1.184	0.788	0.416	0	0



Graph I . Comparison Of Mean Bond Strengths At 24hrs And After 25 Days Of Bracket Bonding Using T Test



Graph II. Time Dependent Release of Antimicrobial Agents

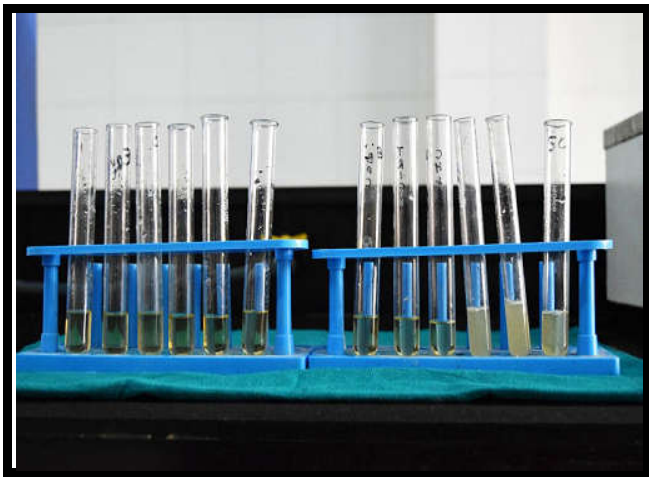


Figure 1. Testing the Minimum Inhibitory Concentration of antimicrobial agent

Figure 2. Tooth mounted on acrylic mould after bonding

The leaching of the antimicrobial agent after immersion in artificial saliva was determined with spectrophotometric analysis after 24hrs of bonding and at 5th day, 10th day, 15th day, 20th day and 25th day after bonding (Table IV and Graph II).

Figure 3. Lawn culture of *S mutan* sprepared on Blood Agar plate to find out the zone of inhibition

Figure 4. Modified composite tablet placed at the centre of the agar plate which was incubated for 24 hrs at 37°C

Figure5: Modified composite placed in the tryptic soy broth and checked for bacterial growth after incubating for 24 hours at37°C

DISCUSSION

It is generally accepted that the insertion of fixed orthodontic appliances creates stagnation areas for plaque and makes tooth cleaning more difficult. The irregular surfaces of brackets, bands, wires and other attachments also limit naturally occurring self-cleansing mechanisms, such as the movement of the oral musculature and saliva.[95]

Figure 6. Different groups of teeth aged by immersion in artificial saliva

Figure 7. UV Double Beam Spectrophotometer (Systronics)

Figure 8. Universal Testing machine (Lloyd instruments) with computer console for recording readings of bond strength testing.

Clinicians have long felt the need for developing effective preventive regimes to avoid demineralization in patients with fixed orthodontic appliances. Recent investigations of preventive strategies in modern adhesive dentistry have focused particularly on various bio-active, antibacterial agents in orthodontic adhesivess.[34,96,97] An ideal action of anti – microbial agent for the purpose of preventing white spot lesions should be independent of patient cooperation, and the drug should be released over a prolonged period of time. In addition, it would be beneficial if the anti-microbial agent were site specific to those areas most susceptible to demineralization, namely, adjacent to bonded orthodontic brackets. The bond strength testing in the present study was done after 24 hours of bracket bonding.

This is in accordance with the results of Yamamoto et al[116] who demonstrated that shear bond strengths of the cured orthodontic adhesives significantly increase till 24 hours and marginally thereafter. The reason for the increase of the bond strength was most probably due to the ongoing post polymerization process within the resin[117]. In the present study the shear bond strengths of the resin tested after 24 hours were 12.60 MPa for the control group, 11.29 MPa for the Benzalkonium chloride modified composite, 10.63 MPa for the triclosan-modified composite group, and 11.31 MPa for the chlorhexidine-modified group (Table I). Although the bond strengths of the modified resin groups were smaller than the control group, the differences were not statistically significant. The bond strength values were sufficiently higher than the 5.7 Mpa recommended by Reynolds[118] as adequate for orthodontic purposes. The decrease in the bond strength of the modified adhesive after 24 hrs, when compared to control, in the present study were in accordance with Othman et al[33] who found a similar decrease after addition of benzalkonium chloride. Shear and tensile bond strength testing of the second set of teeth was done after 25 days of storage in artificial saliva to evaluate the effects of aging on the physical property of the orthodontic adhesives. Nagel[119], (1975) tested specimens at 24 hours and 1 month and concluded there was no deterioration in bond strength. Reynolds and von Fraunhofer[120], (1976) tested specimens at intervals between 3 hours and 6 months. They stated that the bond strength did not vary significantly.In the present study, when the results were compared with the shear bond strengths of the first set of teeth tested after 24 hours, a decrease in bond strength was found in the control group (from 12.60 to 11.39 MPa) (Table II).

This can be explained based on the findings of Soderholmet al[117] and Meng et al[121]. They suggested that composite resin when immersed in water tend to undergo hydrolytic degradation. Chemical degradation of dental composites can be caused by hydrolysis and/or enzyme catalysis from saliva and enzymes in the oral environment, weakening the composite material sufficiently to reduce restoration longevity and mechanical properties (Ferracane[122], 1995, 2006; Geurtsen[124], 1998; Santerre*et al*[125], 2001). Degradation changes the composite microstructure by forming pores or openings from which degradation products, residual monomers, and oligomers can be released. (Goperfich[126], 1996; Geurtsen[124], 1998). In the present study, bond strengths of modified composites were enhanced, (Benzalkonium chloride 11.29 to 12.65, Triclosan 10.63 to 11.09 and Chlorhexidine 11.31 to 11.51), though not significantly after 25 days. This can be explained based on the chemical composition of the composite resins. This was in accordance to the study conducted by…….who reported that the protection of the ester groups in dental composites against hydrolysis may reduce the release of compounds[101].The incorporated anti-bacterial agent may act as filler particle and reduce the chemical degradation of composite resin while enhancing its bond strength. In this study, the results of the Weibull analysis showed that addition of antimicrobial agents to the orthodontic adhesives lowers the characteristic strength (normalizing parameter) of all the modified composite groups compared with the control group (Table III). The reduction in strength may be due to decrease in the number of mechanical sites for attachment of the orthodontic adhesives. When the bond strengths of resin tested after 25 days (Table II) were compared with those tested at 24 hours, the benzalkonium chloride modified group's characteristic strength increased (from 12.17 to 12.36 MPa).

Its Weibull modulus also increased (from 6.03 to 7.12); this indicates better dependability or clinical reliability of the material. For the triclosan-modified group, Weibull modulus increased (from 6.54 to 15.67) however its characteristic strength decreased slightly (from 11.37 to 10.67 MPa). For the chlorhexidine-modified group also, Weibull modulus increased (from 5.77 to 9.02) however its characteristic strength decreased (from 12.21 to 10.97 MPa). This means both have failure rate that increases with time, but strengths were reduced. The MIC values for Benzalkonium chloride, chlorhexidine and triclosan were found out to be 0.12 µg/ml, 0.25µg/ml and 0.24µg/ml respectively. No zones of inhibition were observed when teeth bonded with modified adhesives were placed in the blood agar culture of streptococcus mutans. The diffusion of these chemicals is different in different media which may be the reason for no leaching in agar media[135]. When each bonded tooth was incubated in tryptic soy broth inoculated with Streptococcus mutans, the clarity in the liquid was seen only in the case of benzalkonium chloride. This indicated the efficacy of benzalkonium chloride in short term release and action, as the clarity was due to its bactericidal action. This result confirms the spectrophotometric essay where release of benzalkonium chloride was much higher than its MIC in the first 24 hours (Table IV, Graph II). Table IV showed the spectrophotometric leaching of anti-microbial substances when immersed in artificial saliva. The artificial saliva, which has been used extensively in caries research, was made with deionized distilled water and contained 20 mmol/L NaHCO3, 3 mmol/L NaH2PO4: H2O and 1 mmol/L CaCl2, the proportions found in human saliva). The results showed Benzalkonium Chloride release, as evidenced by spectrophotometric leaching, to be continuous and constant over time, making it potentially desirable for clinical use. The results are in accordance with the study by

Othman et al[33] where the leaching from modified composite samples showed that antimicrobial activity increased with higher benzalkonium chloride content. However, this is not in agreement with the study of Kayo Saito et al[110] where the antibacterial activity of benzalkonium chloride -incorporated resin samples decreased significantly.The results of our study were in accordance with another study Ribeiro and Ericson[136], (1991) where, although initially strong, the antibacterial effect of chlorhexidine did not last for long periods. For the spectrophotometrical experiments only a minor portion of chlorhexidine was released (0.3-5%) from the amount added to the test tablets. This might be explained by chlorhexidine forming insoluble salts whenever combined with silicates and phosphates[104]. Solubility in water affects the release of the agent and triclosan remains relatively insoluble[137]. Therefore, triclosan incorporation in composite might be better when compared with Benzalkonium chloride and chlorhexidine in the long run because of its non-releasing nature. This is in accordance with the study done by Sajad Sainulabdeen et al[138] (2010) concluded that 2.5% Triclosan showed more antimicrobial activity than 2.5% chlorhexidine against Lactobacillus acidophilus and Streptococcus mutans. The proposed mechanism of action of triclosan suggests the material to be an immobilized bactericide which does not leach out of the carrier material, thereby favouring long term anticariogenic activity[64]. Clinical conditions may significantly differ from an in-vitro setting. It needs to be emphasized that this in-vitro study and the test conditions have not been subjected to the rigors of the oral environment. Hot and humid conditions of the oral cavity are highly variable.

Because of the probably differences between in-vivo and in-vitro conditions, the results of this study cannot be extrapolated to the in-vivo. Secondly, the antimicrobial activity of the antimicrobial agents was assessed against only one bacterium – streptococcus mutans. However, there are many other bacteria that are implicated in the etiology of the dental caries. So by seeing all these limitations this in vitro study ,it requires further investigation for more significant clinical results.

Conclusion

- The addition of Benzalkonium chloride to the orthodontic adhesive enhanced its antimicrobial properties to clinically significant levels as compared to addition of Triclosan and Chlorhexidine showed to non-significant levels.
- There was no significant effect on the shear bond strength of orthodontic adhesive modified with the addition of Benzalkonium chloride, Triclosan and Chlorhexidine. However, when compared to control group, there was slight increase in the bond strength of all orthodontic adhesive modified groups probably due to the ongoing post polymerization process within the composite resin.
- Benzalkonium chloride-modified adhesive showed a much higher release of benzalkonium chloride than its Minimum inhibitory concentration level and was found out to be effective in initial stages. Triclosan showed minimal initial release and was found out to be suitable for long duration use because of its non-releasing nature.

Benzalkonium chloride modified adhesive showed better dependability and clinical reliability as compared to Triclosan modified orthodontic adhesive group and Chlorhexidine modified orthodontic adhesive group which was evident with its increased Weibull modulus and increased characteristic strength.

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