



“A Comparative Evaluation of Antimicrobial Activity and Tensile Bond Strength of Orthodontic Adhesive Incorporated With Bioactive Glass and Octenidine Dihydrochloride Antimicrobial Agents: An In Vitro Study”

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ABSTRACT

Background: The goal of this research is to investigate whether incorporating Bioactive glass & Octenidine dihydrochloride antimicrobial agents into widely viable orthodontic adhesive would contribute to antimicrobial activity without compromising the adhesives tensile bond strength.

Methods: 120 adhesive discs and 120 extracted human maxillary premolars were used in the study. These specimens were categorized into 3 distinct groups of 40 each and evaluated at 4 different time intervals i.e., 24 hours, 3 months, 6months, 1 year.

Results: At 24 hours, 3 months and 6 months, it was observed that the antimicrobial activity of bioactive glass is higher than octenidine dihydrochloride. At 1 year, both bioactive glass and octenidine dihydrochloride showed no antimicrobial activity. The tensile bond strength was greater in the control group than with BAG and ODH group at all the time intervals. But both BAG and ODH had sufficient bond strength values.

Conclusion: In conclusion, the newer experimental adhesives incorporated with Bioactive glass and Octenidine dihydrochloride are favorable for clinical application as these have promising antibacterial activity without compromising the orthodontic adhesives tensile bond strength.

Keywords: Bioactive glass, octenidine dihydrochloride, Tensile bond strength, antimicrobial activity.

I. INTRODUCTION

The success of orthodontic treatment lies in correction of pre-existing conditions without damaging the health of the teeth and the tissues that support them. Fixed orthodontic appliances such as brackets and bands act as retentive areas because of their complex designs that favor an accumulation of bacteria, particularly *Streptococcus mutans* and *Lactobacilli*.¹

In orthodontics, composites are frequently

employed for bonding brackets. These composites also act as source for the aggregation of bacteria which play a key role in enamel demineralization. An increase in the colonization of *Streptococcus mutans* decreases the pH which results in demineralization which in turn leads to the progress of white spot lesions (WSL) which represent an early form of enamel caries.²

Enamel demineralization can occur in up to 50% of patients after fixed orthodontic therapy. As a result, numerous researchers introduced an innovative approach to incorporate antibacterial agents into composite resins in minute quantities to resist a microbial attack without altering the physical characteristics of the material, to overcome the development of WSL. Various types of antimicrobial agents have been used, of which most used include leachable agents, polymerizable agents, and antibacterial filters.

Leachable agents are typically water-soluble and are released into a local area under oral conditions. Polymerizable agents copolymerize with resin matrix and offer long-lasting antibacterial protection. Antibacterial filters are metal, metal salts, or oxides that are water insoluble.³ Orthodontic bonding methods that distribute antimicrobial agents to neighboring areas, according to Korbmacher et al⁴, are beneficial because they lessen the requirement for patient compliance and perhaps reduce decalcification.

The introduction of Octenidine dihydrochloride and Bioactive glass as antimicrobial agents is incredibly encouraging. Therefore, analysis of antimicrobial activity and its impact on bond strength would be essential for achieving knowledge of their clinical reliability.



II. METHODOLOGY

STUDY GROUPS:

In this study, samples were categorized into three groups of forty each and evaluated at four different time intervals of ten per interval for antimicrobial activity and tensile bond strength.

- Group 1- Control using commercially available 3M TransbondXT.
- Group 2- Bioactive glass incorporated into 3M TransbondXT.
- Group 3- Octenidine dihydrochloride incorporated into 3M TransbondXT.

Bioactive glass of 15% w/w and Octenidine dihydrochloride of 6% w/w concentrations were incorporated into commercially available photo-activated adhesive. The adhesive and antimicrobial agents were weighed with an electronic digital weighing unit. The adhesive was blended using Speed Mixer (DAC 150.1 FVZ-K) and a homogenous mix was obtained. The adhesive specimens incorporated with antimicrobial agents were called modified adhesives.

Each tooth was individually embedded into self-cure acrylic resin poured in one inch long and one-inch-wide PVC pipe after thorough cleansing under tap water. Teeth were embedded individually in the acrylic mold with the buccal surface parallel to the mold base.

The facial surfaces of teeth were then cleaned with a rubber cup and pumice before bonding. Etching the surface to be bonded was done using 37% phosphoric acid for 15 sec and rinsed with water for 15 sec and air-dried till a white frosty patch appeared. Stainless steel brackets (3M) were then bonded onto buccal surface of the teeth using modified composites. Initially, a light force was applied on the bracket and after proper positioning, a greater force was applied to remove the excess flash and then cured for 40 sec (10 sec on each side of bracket).

A universal testing equipment was used to test the tensile bond strength at a cross-sectional speed of 1mm per minute. Universal joints were used to ensure the proper alignment of the samples to the machine. The bond strength was evaluated at time intervals of 1 day after bonding (T1), 1 month after bonding (T2), 6 months after bonding (T3) and 12 months after bonding (T4). The brackets bonded with modified adhesives were placed in distilled water until evaluation. The results were then recorded in mega pascals.

Streptococcus mutans was used as a test bacterium grown in brain heart infusion broth at 37 °C. Round molds were utilized in the fabrication of adhesive discs of uniform size of 8 mm diameter and 2mm thickness.

The adhesive was blended with Bioactive glass at a concentration of 15% and Octenidine dihydrochloride, of 6% concentration using a Speed Mixer (DAC 150.1 FVZ-K). The concentrations were prepared by pre weighing 60mg of Bioactive glass and 24 mg of Octenidine dihydrochloride and were mixed in 4 gms of composite each.

The modified adhesive was then injected into the molds and excessively filled and firmly pressed against two microscopic glass slides. The discs were polymerized for 60 sec (30 sec from the top and 30 sec from the bottom) with the conventional LED light source. The discs were then separated and stored at room temperature in distilled water until evaluation.

To test the antimicrobial activity the discs were removed from distilled water, air-dried, and tested using an agar disc diffusion assay. For this assay, Brain heart infusion agar was inoculated with 80 microlitres of *Streptococcus mutans* from a 60-fold dilution of 0.5 optical density at 550 nm prepared from a 24-hour culture.

The inoculums were distributed uniformly on an agar plate using a glass rod. The plates were then incubated for 48 hours at 37 °C. After 48 hours the inhibition zone around each sample was measured using a caliper and values were recorded. One agar plate was used to test 3-4 adhesive discs. A total of 120 discs were categorized into 3 different groups of 40 each containing Bioactive glass, Octenidine dihydrochloride, and without any antimicrobial agent as control group. The time intervals of testing include day 1 (T1), 1 month (T2), 6 months (T3), 12 months (T4) with ten samples per time interval.

III. STATISTICAL ANALYSIS

The measurements obtained from the Agar disc diffusion assay and Universal testing machine were tabulated and following statistical analyses were carried out: Arithmetic Mean, Standard Deviation, One way ANOVA, Tukeys multiple posthoc test, Dependent t test.



IV. RESULTS

Table I : Comparison of three groups (control, BAG and ODH) with mean tensile bond strength at different time points (T1, T2, T3, T4) by one way ANOVA

Time points	Sources of variation	Sum of Squares	Df	Mean Square	F-value	P-value
T1	Between Groups	89.29	2	44.64	3.2290	0.0550
	Within Groups	373.35	27	13.83		
	Total	462.63	29			
T2	Between Groups	19.51	2	9.76	1.2030	0.3160
	Within Groups	219.05	27	8.11		
	Total	238.57	29			
T3	Between Groups	22.50	2	11.25	1.5960	0.2210
	Within Groups	190.29	27	7.05		
	Total	212.79	29			
T4	Between Groups	160.59	2	80.30	4.9890	0.0140*
	Within Groups	434.53	27	16.09		
	Total	595.12	29			

*p<0.05

Graph I : Comparison of different time points (T1, T2, T3, T4) with mean tensile bond strength in three groups (control, BAG and ODH)

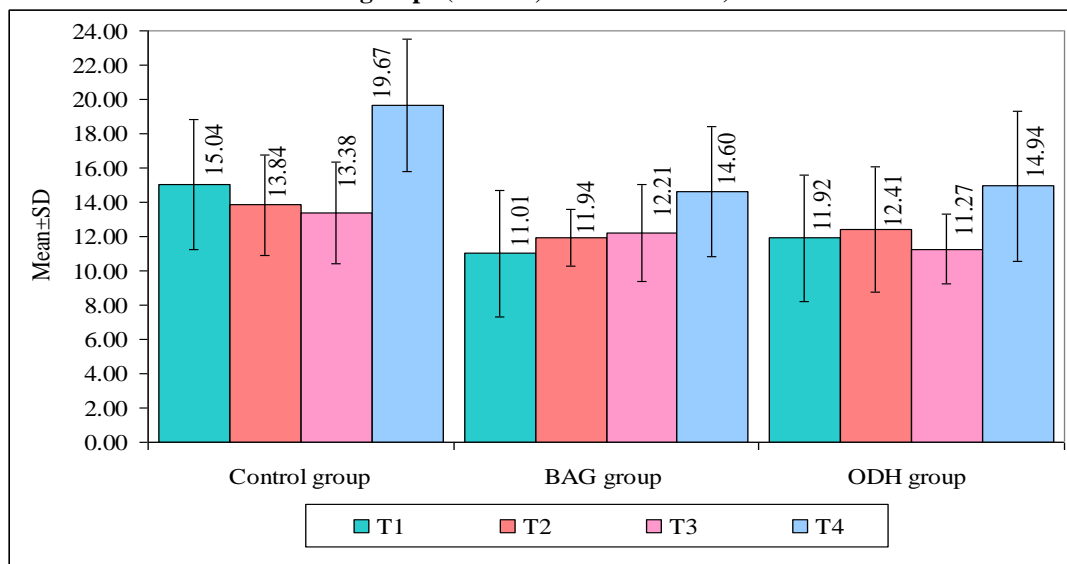


Table II : Comparison of three groups (control, BAG and ODH) with mean antimicrobial activity at different time points (T1, T2, T3, T4) by one way ANOVA

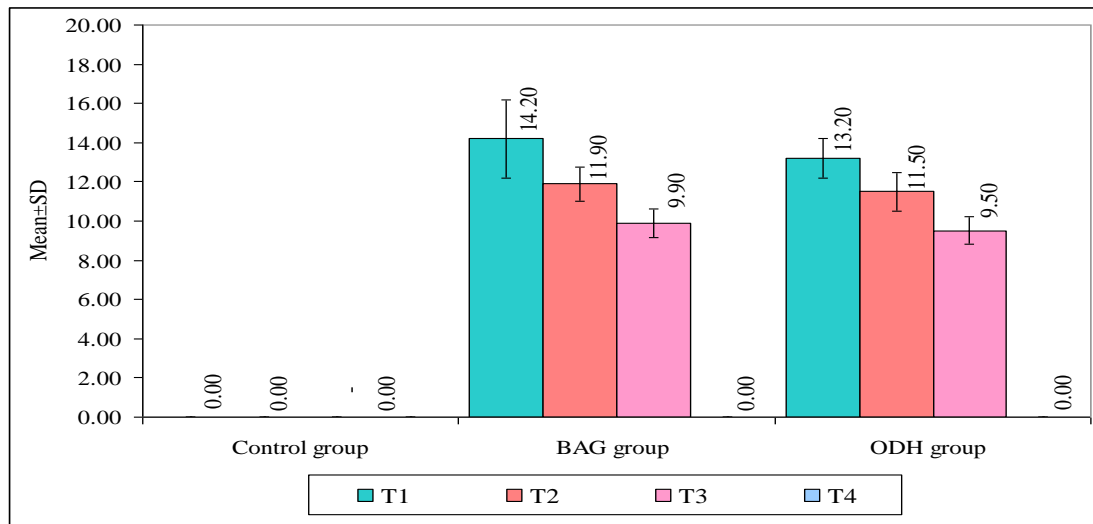
Time points	Sources of variation	Sum of Squares	df	Mean Square	F-value	P-value
T1	Between Groups	1256.27	2	628.13	375.2120	0.0001*
	Within Groups	45.20	27	1.67		
	Total	1301.47	29			



T2	Between Groups	913.40	2	456.70	800.7080	0.0001*
	Within Groups	15.40	27	0.57		
	Total	928.80	29			
T3	Between Groups	628.07	2	314.03	902.0110	0.0001*
	Within Groups	9.40	27	0.35		
	Total	637.47	29			
T4	Between Groups	--	--	--	--	--
	Within Groups	--	--	--		
	Total	--	--			

*p<0.05

Graph II: Comparison of different time points (T1, T2, T3, T4) with mean Antimicrobial activity in three groups (control, BAG and ODH)



In this study, mean tensile bond strength of the Control group at T1 was 15.04 Mpa, that of BAG group was 11.01 Mpa and ODH group was 11.92 Mpa. The mean tensile bond strength of the Control group at T2 was 13.84 Mpa, that of the BAG group was 11.94 Mpa and ODH group was 12.41 Mpa. The mean tensile bond strength of the Control group at T3 was 13.38 Mpa, that of BAG group was 12.21 Mpa and ODH group was 11.27 Mpa. The mean tensile bond strength of the Control group at T4 was 19.67 Mpa, that of the BAG group was 14.60 Mpa, and the ODH group was 14.94Mpa.

No significant difference was found among the study and control groups with respect to the tensile bond strength at T1 (F=3.2290,

p=0.0550)(24 hours), T2 (F=1.2030, p=0.3160)(3 months), T3(F=1.5960, p=0.2210)(6 months). A significant difference was found between three groups (control, BAG and ODH) with mean tensile bond strength at T4 (F=4.9890, p=0.0140) i.e., 1 year (Table I, Graph I).

In terms of antimicrobial activity, a significant difference was seen among the study and control group at T1 (F=375.2120, p=0.0001), T2 (F=800.7080, p=0.0001), T3 (F=902.0110, p=0.0001). However, this difference ceased to exist between the three groups (control, BAG and ODH) with mean antimicrobial activity at T4. (Table II, Graph II).



V. DISCUSSION

The branch of clinical orthodontics was revolutionized with an innovative idea of incorporating antibacterial agents into orthodontic bonding adhesives to prevent WSLs. Since then, several attempts have been made to improve this protocol and various experimental formulations were used for the reduction of bacterial growth.

In orthodontics, the bond that is formed between the surface of the tooth and bracket should be high enough to not only hold onto the surface of the tooth but also to resist any accidental debonding of the bracket during treatment. The other side of the coin is that the bond formed should also be just enough to give into optimal debonding forces without causing any enamel fractures after the completion of treatment.

Reynolds (1975) suggested that bond strength of about 6 to 8 Mpa would be adequate between the metal bracket and the enamel surface⁵.

Meng et al⁶ indicated that when composite resins are immersed in water, they tend to hydrolytically degrade, and that the longer the immersion duration, poorer the bond strength. The tensile bond strength was examined rather than shear bond strength in this study because it provides more consistent results and is less sensitive to minor misalignment errors⁷. The bond strength of any adhesive usually changes with time. In general, any given adhesive takes around 24 hours to be cured completely. In the current study, tensile bond strengths were measured at four different periods as the course of orthodontic treatment is generally prolonged and ranges from one to two years.

As seen in Table I, comparison of three groups (control, BAG, and ODH) with mean tensile bond strength at different time points (T1, T2, T3, T4) by one way ANOVA showed no significant difference between the three groups (control, BAG and ODH) with mean tensile bond strength at T1, T2, T3 ($F=1.5960$, $p=0.2210$). It means that the mean tensile bond strength is similar in three groups (control, BAG, and ODH) at T1, T2, T3. A significant difference between the three groups (control, BAG, and ODH) with mean tensile bond strength at T4. It means that the mean tensile bond strength is different in three groups (control, BAG, ODH) at T4 with control group higher than BAG and ODH group. Hence the addition of BAG and ODH into orthodontic adhesive did not have a negative impact on the tensile bond strength of the orthodontic adhesive.

Results in present study were similar to

previous studies, Hannanh Ghadirian et al⁸ assessed the shear bond strength of adhesive with quaternary ammonium salts and found no adverse effects on bond strength. S.Imazato et al⁹ conducted a study to assess the antibacterial effect of orthodontic adhesive with 12-Methacryloxydodecylpyridinium bromide (MDPB) and its effects on physical properties of adhesive and found that incorporation of MDPB into orthodontic adhesive had no impact on the bond strength of the orthodontic adhesive.

Chanjyot Singh et al¹⁰ used 2.5% chlorhexidine with light cure composite and tested the shear bond strength of modified composites, results showed no adverse effect on bond strength of the modified adhesives.

In contrast, certain studies showed a decrease in bond strength after incorporating antimicrobial agents. In a study done by, Amir Hossein Mirhashemi et al¹¹ shear bond strength was reduced with use of antimicrobial photodynamic therapy with methylene blue and indocyanine green. Mona Riad et al¹² evaluated shear bond strength of adhesive containing silver nanoparticles and found a decrease in bond strength by the addition of silver nanoparticles into the orthodontic adhesive.

In the current research, antibacterial activity of modified adhesives was assessed by agar disc diffusion assay among the Control, BAG, ODH groups at time intervals of day 1(T1), 1 month(T2), 6 months(T3), 12 months(T4). Generally, as all adhesives get completely polymerized within 24 hours, evaluation at Day 1 and further after 1month, 6 months, 12 months were done. Tahani Musallam et al¹³ evaluated the antimicrobial property of Cetylpyridinium Chloride where in samples were tested for 196 days. In our study samples were evaluated for 12 months to assess the long-term effects of antimicrobial agents.

In this study, it was noted that a considerable difference between three groups (control, BAG, and ODH) with mean antimicrobial activity at T1, T2, T3 was noted, and all the three groups showed no antimicrobial activity at the T4 time interval as seen in Table II. The antimicrobial activity of Control group was 0 mm at T1, T2, T3, T4. The mean antimicrobial activity of BAG group was 14.20 mm at T1, 11.90 mm at T2, 9.90 mm at T3, 0 mm at T4. The mean antimicrobial activity of ODH group was 13.20 mm at T1, 11.50 mm at T2, 9.50 mm at T3, and 0 mm at T4.

Both BAG and ODH groups showed similar antimicrobial activity with the BAG group



slightly higher antimicrobial activity compared with the ODH group. Hence both BAG and ODH are effective as antimicrobial agents in the reduction of bacterial growth during orthodontic treatment.

Results obtained in this current study were in accordance with previous studies done using other antimicrobial agents. Imazato et al⁹ observed that the use of an antibacterial monomer (2.5% MDPB) in an adhesive resin reduced bacterial growth.

In a study conducted by Tahani Musallam et al¹³ cetylpyridinium chloride, an antiplaque agent was used as a bactericidal agent in the orthodontic adhesive at concentrations of 2.5%, 5% and 10% and results of the study concluded it as effective in antibacterial activity. Sug Joon et al¹⁴ used silver nanoparticles, Maryam Poosti et al¹⁵ used titanium dioxide nanoparticles. Both studies reported a reduction in bacterial growth and effectiveness as a bactericidal agent in the orthodontic adhesive.

It was however noted in this study that antimicrobial activity was sustained only for 6-8 months. Hence adjunctive methods such as fluoride mouth rinses, varnishes, toothpaste, and gels can be recommended as adjuvant antimicrobial aids.

VI. LIMITATIONS

One of the significant limitations, as observed with an in vitro study, is the difficulty of accurate reproduction of the environment in the oral cavity. Despite the efforts that are put forth to produce an oral environment, it remains a practical impossibility.

There's no way of knowing how long the system's antimicrobial action will last, especially in the oral environment. Numerous variables are associated with bond strength tests, which make them technique sensitive. It means that the same study when performed by different operators or under different test conditions produces varied results. Furthermore, the presence of saliva of the patient might alter the results when performed intraorally.

VII. CONCLUSION

The conclusions drawn from the current study were listed herewith

➤ The amalgamation of Bioactive glass and Octenidine dihydrochloride into composite adhesive material added antimicrobial properties with inhibition in the bacterial growth.

- The addition of these antimicrobial agents had no adverse effect on the tensile bond strength of the orthodontic adhesive.
- When compared between Bioactive glass and Octenidine dihydrochloride, Bioactive glass is superior to Octenidine dihydrochloride as it is a polymerizable agent while Octenidine dihydrochloride is a leachable chemical compound.
- The long-term antimicrobial activity of both agents was sustained only for a period of 6-8 months. Hence adjunctive methods such as fluoride mouth rinses, varnishes, toothpaste, and gels should be used.

It can be said that, the newer experimental adhesives incorporated with Bioactive glass and Octenidine dihydrochloride are favorable for clinical application as these have promising antimicrobial activity without altering the tensile bond strength of the orthodontic adhesive.

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