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Evaluation of antibacterial effect of methylene blue-mediated photodynamic therapy on orthodontic brackets and modules: an in-vitro study

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ABSTRACT: This study investigates the effectiveness of Methylene blue-mediated antibacterial photodynamic therapy (APDT) in reducing Streptococcus mutans accumulation on orthodontic appliances. A photodynamic coating was applied to orthodontic brackets and modules using a photo-crosslinking polymer, Poly (vinyl alcohol)-Stilbazole Quaternized, combined with methylene blue (MB). The samples were divided into four groups: coated brackets (CB), uncoated brackets (UCB), coated modules (CM), and uncoated modules (UCM). After UV-A light and red light (660nm) activation, the samples were exposed to a bacterial solution, with bacterial growth assessed at 1, 2, and 3 weeks. Results demonstrated a significant reduction in bacterial growth on the coated samples, with reductions of 99%, 98%, and 95% for CM and 98%, 97%, and 95% for CB across the three weeks, respectively. While the tensile strength of the coated appliances decreased slightly, it remained statistically insignificant. These findings suggest that photodynamic coating can be a promising solution to control bacterial accumulation around orthodontic appliances.

KEYWORDS: Antibacterial Photodynamic therapy, Photodynamic coating, Orthodontic Brackets, Methylene Blue, Streptococcus mutans.

I. INTRODUCTION

Orthodontic treatment requires the placement of fixed or removable appliances onto the tooth surfaces. This inevitably changes the oral hygiene needs of the patient undergoing treatment. The placement of brackets results in new surfaces and niches where bacteria can accumulate. The brackets, wires, elastomeric modules and temporary anchorage devices create a more conducive environment for bacteria by easily trapping food particles and plaque consequently leading to rise in the total amount of microbes in the oral cavity.(1–4) As a result, specific types of bacteria found in dental plaque, like Streptococcus mutans and Lactobacilli, might rise.

Additional oral hygiene practice is essential to manage biofilm during orthodontic treatment. Maintaining hygiene involves brushing, mouthwash, and flossing, which require patient participation, and not adhering to the routine can result in white spot lesions and other oral infections. In response to these concerns, antibacterial photodynamic therapy has emerged as a promising adjunctive treatment option.(5,6) It involves the application of a photosensitizing agent, typically a non-toxic dye like methylene blue or toluidine blue to the teeth and gingiva.(7–9)

When exposed to a specific wavelength of light, the photosensitizer becomes activated and generates reactive oxygen species (ROS). These reactive oxygen species induce microbial cell death by damaging bacterial membranes and intracellular structures, thereby effectively reducing bacterial load. The application of antibacterial photodynamic therapy (APDT) in orthodontics offers several advantages. It provides a non-invasive and targeted approach to bacterial control, complementing traditional oral hygiene practices; also, it provides self-sterilization properties to fixed orthodontic appliances.

Additionally, APDT has shown potential in reducing the incidence of white spot lesions (early signs of dental caries) and improving periodontal health during orthodontic treatment.(6,10,11) As research continues to explore the efficacy and safety of antibacterial photodynamic therapy in orthodontics, its integration into clinical practice holds promise for enhancing treatment outcomes and maintaining oral health throughout the orthodontic journey.(12)

The purpose of the in-vitro research was to assess how effective methylene blue-mediated photodynamic therapy is when applied as a coating on orthodontic brackets and modules, and to

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determine if it causes any changes in the physical properties of elastomeric modules.

II. MATERIAL AND METHODS

The study was approved by the Institutional Ethical Committee under the number 244/IEC/SS/202. The sample size was estimated assuming a power of 80 % in this case, a total of 21 brackets and 21 elastic modules per group (total of 84 samples) were needed to achieve maximum power with an alpha level set at 0.05.

The brackets (3M Unitek Gemini twin brackets) and modules (3M Unitec AlastiK) were grouped into four groups of 21 each: Group 1: Coated Brackets (CB), Group 2: Uncoated Brackets (UCB), Group 3: Coated Modules (CM), and Group 4: Uncoated Modules (UCM). All the samples were sterilized in the autoclave for 15 minutes at 121°C.

The formulation of the of photodynamic coating followed the protocol of Ghareeb et al.(13) for preparation of the coating a photocrosslinkable polymer stilbazol quaternized polyvinyl alcohol (SbQ-PVA) (Polysciences, India) was used as the base for the coating. Deionized water and polymer were mixed in a 10:1 ratio until a uniform solution was achieved, as assessed by the unaided eye (Figure 1). Methylene blue was added to this aqueous SbQ-PVA at 1% photosensitizer loading (Figure 2). The solution was transferred to the airbrush, and spraying was done over the experiment samples. The mesh of the orthodontic brackets was not covered (Figure 3).

The coating was light-cured using UV light (MelodySusie 36W UV Lamp Light) for 60 minutes. The samples were washed gently to remove any residual uncured polymer. Another layer of coating was applied, cured for 30 minutes, and gently rinsed off. Through stereomicroscopy examination of the samples, the homogeneity of the coating was verified. UV light has bactericidal properties, which is why bacterial testing was conducted after photocuring to ensure that any reduction in bacterial growth was due to the photosensitizer. The samples were kept under a red LED light bulb (660nm) for 30 minutes for photo-activation of methylene blue. The distance of coated samples from the light source was 10 cm (Figure 4).

For biofilm testing test strain of Streptococcus mutans in the lyophilized form was obtained from the National Collection of Industrial Microorganisms (NCIM 5660). The bacterial culture was revived and checked for purity before preparing the bacterial solution.

A vortexed bacterial cell suspension of 100µl inoculated to 100mL of BHI broth was prepared. The control and experiment samples were dipped in this suspension and gently swirled. This process was done for both the test samples. After the bacterial biofilm was allowed to adhere to the samples for 30 minutes, the samples were taken out from the bacterial suspension and dipped in 0.9% saline to detach unadhered bacterial cells from the experiment and control group samples.

After removing the detached cells, only the adhered biofilm remained on the samples. This biofilm was then allowed to grow over the test samples for 1 week. These were then kept in 100 mL buffer sodium chloride peptone solution and then vortexed for 60 seconds 3 times to detach the biofilm. A 100µL from each control and experiment group was taken and spread on BHI in duplicates and the incubation process was repeated.

After vortexing, the brackets were gently rinsed with distilled water and reimmersed in the bacterial solution for 30 minutes. The process of removing the bacterial cells and growth of the biofilm over the brackets and modules was repeated; the biofilm was allowed to grow for over 14 days and the same process was carried out for up to 21 days. This was done to check the longevity of the antibacterial effect of the photosensitizer present in the coating. The reduction in antibacterial effectiveness may also be due to photobleaching, a photochemical change in the dye caused by oxidation from free radicals.

To test the tensile strength CM and UCM were mounted on the universal testing machine before being subjected to bacterial testing.

FIGURE 1 HOMOGENOUS SBQ-PVA SOLUTION

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Figure 2 SBQ-PVA/METHYLENE BLUE SOLUTION

Figure 3 PHOTODYNAMIC SPARY COATED USING AIRBRUSH

Figure 4 EXPERIMENT SAMPLES EXPOSED TO RED LED LIGHT FOR PHOTOACTIVATION

III. STATISTICAL ANALYSIS

The data obtained was subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS Version 25; Chicago Inc., IL, USA). Data comparison was done by applying specific statistical tests to find out the statistical significance of the comparisons.

Kolmogorov –Smirnov and Shapiro-Wilk tests were performed to determine the normality of the data for the distribution of Streptococcus mutans colony count in elastomers and brackets. Variables were compared using mean values and standard deviation. The mean for different readings for elastomers and brackets at different time intervals were compared using ANOVA test. An Independent 't' test was applied to find differences between groups. A p-value less than 0.05 was considered statistically significant.

IV. RESULTS

Comparative evaluation of UCM for mean concentration of Streptococcus mutans at various time intervals showed minor fluctuations, starting at 790,000 CFU/ml in week 1, peaking slightly at 802,381 CFU/ml in week 2, and then slightly decreasing to 794,286 CFU/ml by week 3 (Table 1). A p-value of 0.417 implies that there are no significant differences in the bacterial counts over the three weeks. This lack of significant change suggests that the uncoated elastic modules did not exhibit a substantial antibacterial effect against S. mutans throughout the study.

The comparative evaluation of CM for S. mutans over three weeks showed significant changes in bacterial counts, as indicated with a p-value of <0.001, suggesting strong statistical significance. The results suggest a substantial reduction in the bacterial counts from week 1 to week 3, demonstrating the efficacy of the coating on the elastic modules to inhibit S. mutans over time (Table 2). Overall, the data supports the effectiveness of the coated elastic modules in reducing bacterial colonization, critical in orthodontic treatment to minimize the risk of infections.

Assessment of percentage reduction of S. mutans in CM group over three weeks demonstrated significant changes in bacterial reduction. The highest reduction was noted in the first week with an average of 99.5% with a slight decrease in 14 days to 98.2%, still a high percentage but showing a reduction from Week 1. In the 3rd week a more marked decrease in bacterial reduction to 95.4%, was observed (Table 3). This indicates a greater variability in the effectiveness of antibacterial treatment.

The counts of S. mutans on UCB at three different time intervals over three weeks showed that the initial count was relatively high 723,757 CFU/ml at 7 days which increased even more to 806,852 CFU/ml in 14 days and finally, a slight decrease of 791,904 CFU/ml was seen in 21 days (Table 4). A p-value of 0.08, suggested that there was no statistically significant difference in the counts of S. mutans on uncoated brackets over the three weeks. The results indicate that while there was a slight fluctuation in the numbers of bacteria over the weeks, these changes are not statistically significant, suggesting that the uncoated brackets do not show a significant reduction in the bacterial count over time.

The comparative evaluation of S. mutans counts on CB over three weeks showed that the mean bacterial counts increased from 21,476 in week 1 to 33,061 in week 2, finally reaching

53,000 by week 3. Despite the upward trend, the variability in bacterial counts, as indicated by the standard deviations, was quite high each week, reflecting considerable inconsistency in bacterial growth across the sample (Table 5). A p-value of 0.28 indicated that the differences in mean bacterial counts between the time intervals were not statistically significant. This suggested that while there appeared to be a trend of increasing bacterial counts on coated brackets over time, the changes were not consistent enough across the sample to conclude definitively that the coating affects bacterial growth over the periods tested.

The percentage reduction of S. mutans on CB group over three-time intervals showed the mean percentage reduction was 98 ± 1.3% in week 1. There was a slight decrease in the mean percentage reduction to 97.3±1.1% and a further decrease to 95.7% (Table 6). A significant p-value of 0.00 suggests there are statistically significant differences in the mean percentage reductions across the three weeks. The trend indicates a decreasing effectiveness in reducing Streptococcus mutans on brackets over time.

Evaluation of tensile strength:

The orthodontic elastic modules were checked for any change in the tensile strength using IS 3400(Part 1): 2021. The uncoated modules had an average tensile strength of 22.7 MPa. Whereas the coated brackets had an average tensile strength of 19.8 MPa.

Table 1: Comparative evaluation of uncoated elastic modules (UCM) for Streptococcus mutans at various time intervals

Time Intervals	N	Mean	Std. Deviation	95% Confidence Interval	
				Lower bound	Upper bound
Week 1	21	790000.0000	33763.88603	774630.8633	805369.1367
Week 2	21	802380.9524	25080.82174	790964.2992	813797.6056
Week 3	21	794285.7143	32182.51522	779636.4090	808935.0196
'F' statistic	.888				
df	2				
P value	.417 (NS)				

*=Significant; NS=Not Significant

Table 2: Comparative evaluation of coated elastic modules (CM) for Streptococcus mutans at various time intervals

Time Intervals	N	Mean	Std. Deviation	95% Confidence Interval	
				Lower bound	Upper bound
Week 1	21	36271.4286	4989.50327	2543.1536	4294.9416
Week 2	21	14157.1429	2067.98729	13215.8063	15098.4794
Week 3	21	3419.0476	1924.21907	34000.2339	38542.6232
'F' statistic	537.747				
df	2				
P value	.000*				

Table 3: Comparative evaluation of percentage reduction for Streptococcus mutans at various time intervals (CM group)

Time Intervals	N	Mean	Std. Deviation	95% Confidence Interval	
				Lower bound	Upper bound
Week 1	21	99.5661	.24283	99.4556	99.6766
Week 2	21	98.2376	.23089	98.1325	98.3427
Week 3	21	95.4279	.63922	95.1370	95.7189

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'F' statistic	539.912
df	2
P value	.000*

Table 4: Comparative evaluation of uncoated brackets (UCB) for Streptococcus mutans at various time intervals

Time Intervals	N	Mean	Std. Deviation	95% Confidence Interval	
				Lower bound	Upper bound
Week 1	21	723757.1429	216845.37941	625050.3096	822463.9761
Week 2	21	806852.3810	33455.30779	791623.7074	822081.0545
Week 3	21	791904.7619	32034.20791	777322.9653	806486.5585
'F' statistic	2.514				
df	2				
P value	.089				

Table 5: Comparative evaluation of coated brackets (CB) for Streptococcus mutans at various time intervals

Time Intervals	N	Mean	Std. Deviation	95% Confidence Interval	
				Lower bound	Upper bound
Week 1	21	21476.1905	28994.99793	8277.8257	34674.5553
Week 2	21	33061.9048	55119.58342	7971.7712	58152.0383
Week 3	21	53000.0000	93452.79557	10460.7975	95539.2025
'F' statistic	1.270				
df	2				
P value	.288 (NS)				

Table 6: Comparative evaluation of percentage reduction for Streptococcus mutans at various time intervals (CB group)

Time Intervals	N	Mean	Std. Deviation	95% Confidence Interval	
				Lower bound	Upper bound
Week 1	21	98.0474	1.33769	97.4385	98.6563
Week 2	21	97.3430	1.16759	96.8115	97.8745
Week 3	21	95.7568	.80704	95.3895	96.1242
'F' statistic	22.797				
df	2				
P value	.000*				

V. DISCUSSION

Orthodontic treatment with any modality typically lasts a year or more during which it is imperative to maintain plaque plaque-free surface of tooth surfaces and the appliance. Studies confirm that oral microbiota undergoes changes with additional surfaces provided by appliances and there is an increase in plaque retention especially around brackets and elastomeric modules.(2,14–17) Streptococcus mutans, the major inhabitant of the oral cavity and dental plaque is responsible for several dental ailments including white spot lesions, dental caries, gingivitis and periodontitis.(18,19) Previous studies suggest that photodynamic therapy (PDT) as an adjuvant to oral prophylaxis significantly reduces bacterial count for four weeks and more.(20–22)

Methylene blue has been proven effective against both gram-positive and gram-negative bacteria. Methylene blue is a widely known histological dye that belongs to the phenothiazinium class of compounds. MB has an absorption spectrum of 550–700 nm, with a peak at 665 nm, and exhibits bactericidal effectiveness even at a concentration of 0.0001%.(23)

The results obtained in our study suggest that methylene blue-mediated photodynamic therapy effectively reduces the count of Streptococcus mutans by 98% from the bracket surface. These results are in accordance with previous studies which showed methylene blue having bactericidal properties. Alshahrani et al(24) in a study concluded that PDT in adjunct with tongue scraping significantly decreases halitosis in patients undergoing orthodontic treatment. He found a 100% reduction in H2S and a significant reduction in bacteria involved in periodontal diseases with 0.005% methylene blue activated with 660 nm diode LASER. Foggiato A. et al(25) in an experiment, developed a photodynamic inactivation device that used methylene blue against Staphylococcus aureus, S. mutans, and E. coli where the bacterial count significantly reduced, and S. mutans growth inhibition was 100%. Soares L. et al(26) used APDT using methylene blue and toluidine blue as photosensitizers, combined with red LED irradiation, and this significantly reduced colony-forming units (CFU) around orthodontic brackets by approximately 90%.

There have been studies evaluating the antibacterial effect of other photosensitizers with orthodontic brackets. Kamran et al(27) in an in-vitro investigation used riboflavin against S. mutans and S. sanguinis around orthodontic brackets and found riboflavin mediated-APDT to significantly reduce the amount of these bacteria which was assessed by counting CFU and a live/dead ratio using confocal laser microscope imaging. Mocuta et al(28) conducted a study to assess the efficacy of 2% methylene blue and a 5% chlorophyllin-phycocyanin mixture in disrupting Streptococcus mutans biofilms that were artificially formed on orthodontic brackets bonded to extracted tooth samples and found chlorophyllin-phycocyanin mixture to be more effective.

In our study CM showed a significant reduction of Streptococcus mutans which was 99% without significant degradation in its physical properties. Similar results were seen in a study by Ghanemi M. et al(29) who coated elastomeric ligatures with reduced nanographene oxide- nano curcumin (rGO-nCur) and tested its efficacy against S. mutans biofilms which showed effective and significant inhibition by 83.62% of the biofilm with 5% rGO-nCur. To our knowledge, there are no other studies with photodynamic coating applied over elastomeric modules. However, the effect of UV light on the tensile strength of elastic chain has been assessed by Wahab et al(30) which showed that the duration of UV light can have varying effects leading to the degradation of its polymer. In our study, we observed a slight decrease in tensile strength; however, the cause of this reduction whether it is attributable to the additional polymer layer (i.e., SbQ-PVA) or the exposure to UV light remains uncertain.

As research continues to advance, methylene blue PDT holds the potential to become a valuable addition to the armamentarium of antimicrobial strategies, contributing to improved patient outcomes and reduced antibiotic resistance.

CONCLUSION

1. The results confirmed that methylene blue is an effective photosensitizer for Streptococcus mutans inhibition and can be used on orthodontic appliances to provide them with self-sterilization ability.
2. The bactericidal effect remains for up to 3 weeks but decreases over time attributed to photobleaching.
3. The tensile strength of the elastic module does not decrease significantly after coating is applied.

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