RESEARCH PAPER

Comparative evaluation of antimicrobial efficacy of silver oxide nano particles and chlorhexidine mouthwash when used alone and in combination on orthodontic brackets: an In-vitro study

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ABSTRACT

Chlorhexidine (CHX) gluconate is a widely recognized antimicrobial agent in dentistry known for its ability to eradicate oral microbial populations. Silver nanoparticles (AgNP) are effective antimicrobial agents frequently utilized in various medical applications. After fixed orthodontic treatment, enamel demineralization may occur in approximately 50% of patients.

Aim : The aim of this study was to compare the antimicrobial efficacy of 0.2% chlorhexidine mouthwash and silver oxide-coated nanoparticles when used alone and in combination on orthodontic brackets against Streptococcus mutans(S.mutans). S.mutans has been identified as factors that support the development of cariogenic microbiota. The synergistic effect of the two solutions has been evaluated in this study.

Materials and Methods: 30 premolar stainless steel brackets were divided into three groups (A, B, C). Each group consisted of 10 brackets. Brackets in group A was immersed in 0.2% chlorhexidine. Brackets in group B was coated with silver oxide nanoparticles. Brackets in group C was immersed in 0.2% chlorhexidine solution after coating with silver oxide nanoparticles (CHX-AgONP). Brackets then were subjected to microbiological tests for assessment antibacterial properties against S.mutans.

Results: The multiple comparison of mean differences in Colony Forming Units (CFUs) of S. Mutans at a 14-day period reveals the following findings among the three groups. Group C showed significantly least mean CFU counts compared to Group A, with mean difference of 1.595 (95% CI, 0.106 to 3.084) and Group B, with mean difference of 12.337 (95% CI, 10.748 to 13.726) and the mean differences were statistically significant at $p=0.03$ & $p<0.001$ respectively. This was then followed by Group A which showed significantly lesser mean CFU counts as compared to Group B, with mean difference of -10.642 (95% CI, -12.131 to -9.153) and the mean difference was statistically significant at $p<0.001$. This implies that, Group C showed significant reduction in mean CFUs count, followed by Group A and least in Group B at 14 days Incubation period. CHXAgONP combined solution exhibited the highest efficacy in comparison to these solutions used alone.

Conclusion: The present study demonstrates the antimicrobial efficacy of a novel mixture of CHX-AgONP solution, and it may be developed as a promising antimicrobial agent against S.mutans.

Keywords: antimicrobial, chlorhexidine mouthwash, nanoparticles, silver oxide nanoparticles, streptococcus mutans.

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INTRODUCTION

Fixed orthodontic appliances play a vital role in comprehensive orthodontic treatment. They are highly versatile systems that allow to achieve accurate, three-dimensional control of tooth movement. Orthodontic appliances act as plaque-retentive areas, making it challenging to maintain hygiene. As dental plaque contributes to oral disease such as enamel demineralization and gingivitis, it is essential to control and remove it to maintain good oral hygiene [1,2]. The placement of brackets and arch wires can alter the composition of dental plaque within the oral environment. Orthodontic patients typically experience an increase in salivary microbial load following the insertion of brackets and arch wires making them more prone to enamel decalcification, leading to the formation of white spot lesions(WSLs)[3].This risk is linked to local changes in mineral balance adjacent to brackets [4]. The acidity of plaque near orthodontic brackets slows down the remineralization process, leading to possible enamel decalcification. The level of mineralization is closely linked to enamel transparency, so the initial demineralization of the enamel often appears as WSLs, which are commonly seen in orthodontic patients under loose bands, around the border of the bracket base, and in areas that are difficult to clean with a toothbrush and often go unnoticed by the patient [5]. The bacterial flora composition of plaque alters swiftly after the use of orthodontic appliance with a notable rise in the levels of acidogenic bacteria, particularly Streptococcus mutans (*S. mutans*)[6].

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Prior to the placement of the orthodontic appliances, emphasis should be placed on the management of dental plaque. Orthodontic patients are often advised to use chemical mouthwash along with traditional tooth cleaning aids such as toothbrush and floss. It's essential to remind patients that chemical agents are not a substitute for thorough brushing and flossing [7].

Chlorhexidine (CHX) is considered the gold standard for assessing the effectiveness of other antimicrobial agents due to its beneficial outcomes. Its superior antiplaque properties serve as a broad-spectrum disinfectant with antibacterial activity for wide range of microorganisms, encompassing both gram-positive and gram-negative organisms, such as bacterial spores [8]. Despite CHX being tested in clinical trials to eliminate *S.mutans*, the bacteria typically reappear after 2-3 months. There is no definitive evidence of *S.mutans* being completely eradicated from the oral cavity. Additionally, prolonged attempts to eliminate these bacteria using high concentrations of CHX may have detrimental effects on the oral mucosa [9]. Nanoparticles with antibacterial properties can be integrated into orthodontic materials by blending them with composites and glass ionomers or applying them as surface coatings on arch wires and brackets [10].Silver oxide nanoparticles (AgONP) exhibit high levels of antiviral, antibacterial, and antifungal activity against several kinds of viruses and bacteria. Because of their surface charge characteristics, anti-viral, and anti-bacterial qualities, synthesized nanomaterials can be used as preventive measures, potentially helping to treat the development of WSLs [11-12].

Hence, this study was done to compare the antimicrobial efficacy of 0.2% chlorhexidine mouthwash and silver oxide-coated nanoparticles when used alone and in combination on orthodontic brackets after a period of 24 hours and 14 days .

The null hypothesis was that there would be no difference in mean CFU reduction of *S. mutans* at 14 days among brackets treated with CHX, AgO coating, or their combination; the primary endpoint was CFU reduction at day 14.

Materials And Methods

This In-vitro study was conducted in the Department of Orthodontics at The Oxford Dental College, Bengaluru, Karnataka, India, in collaboration with Nanowatts Technologies Pvt. Ltd. (Bangalore ,India) and Azyme Biosciences (Bangalore ,India) from June 2024 to July 2024. Ethical approval was obtained from the Institutional Review Board of The Oxford Dental College (Approval No. TODC/018/ECAL/2022-23, May 5, 2023).The overall study design, sample allocation, and testing sequence are summarized in Figure *1*.

Sample size estimation

The sample size has been estimated using the GPower software (latest ver. 3.1.9.7; Heinrich-Heine-Universität Du¨sseldorf, Du¨sseldorf, Germany). The sample size estimation was performed at 5% alpha error (α =0.05), with an effect size of 58% & the power of 80%, revealed that a minimum of 30 samples will benecessary for the present study. So, each study group will consist of 10 samples [10 samples x 3 groups = 30samples].

This study was done on 30 specimens of stainless steel preadjusted edgewise appliance premolar brackets(JJ orthodontics Private Limited, Kerala, India). The specimens were divided into three groups. Each group consisted of 10 brackets. Brackets in group A was immersed in 0.2% chlorhexidine(Chlohex,Dr. Reddy's Laboratories Ltd.Hyderabad, India). Brackets in group B was coated with silver oxide nanoparticles. Brackets in group C was immersed in 0.2% chlorhexidine solution after coating with silver oxide nanoparticles (CHXAgONP).(Figure 2- 3)

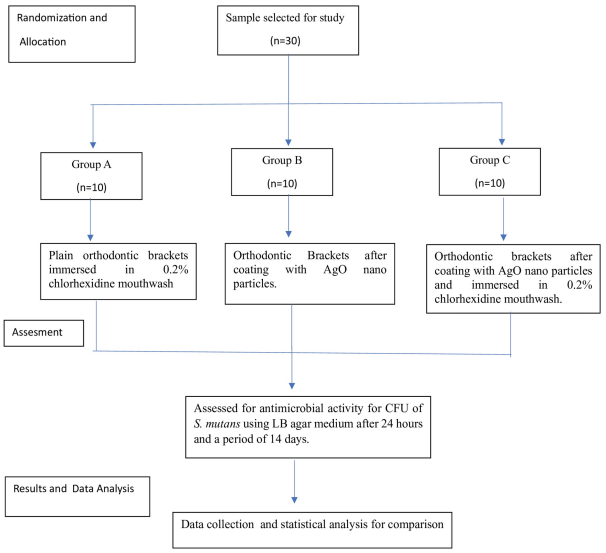
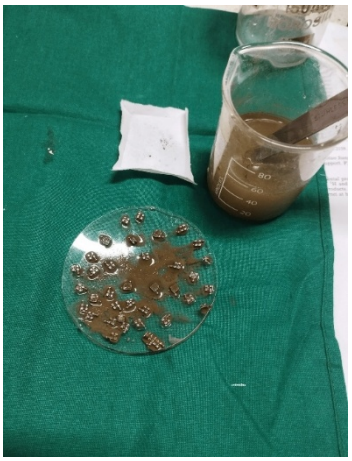


FIGURE 1: Study flowchart depicting sample selection, randomization, and assessment procedures.



AgO- silver Oxide nanoparticles, CFU- Colony Forming units, *S.mutans* - Streptococcus mutans

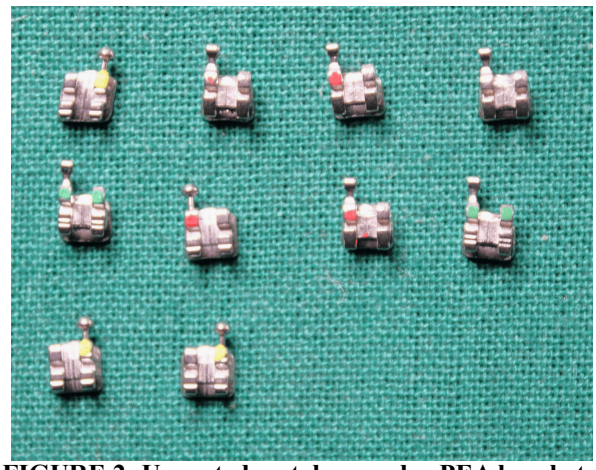


FIGURE 2: Uncoated metal premolar PEA brackets
PEA - pre adjusted edgewise appliance

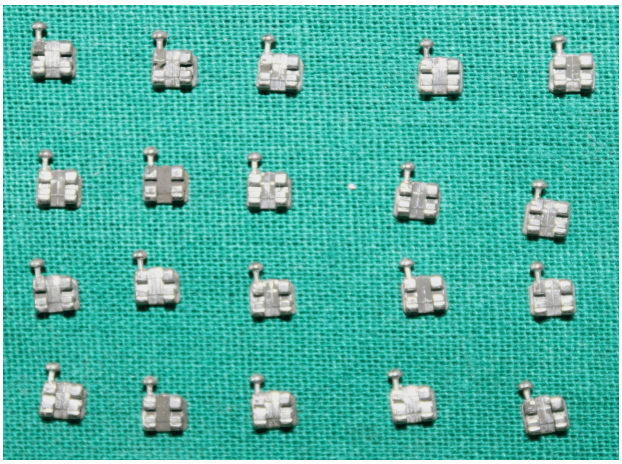
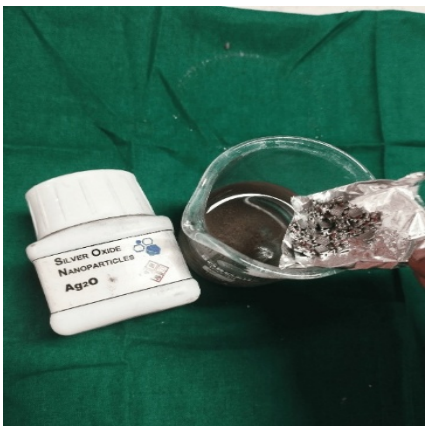


FIGURE 3: Metal premolar PEA brackets coated with silver oxide nanoparticles
PEA-pre adjusted edgewise appliance

Preparation of silver oxide-coated orthodontic brackets
Surface modification of stainless steel orthodontic brackets with AgO was carried out using Sol-Gel dip method described as by Syed SS et al. [13] in Nano Watts Technology, Bengaluru . Premolar brackets were subjected to cleaning with ethanol in an ultrasonic cleaner for ten minutes before being rinsed with distilled water. Afterward, a nitrogen stream was utilized to dry the brackets. Synthetic silver oxide (AgO; CAS No. 20667-12-3,99% purity, NanoResearch Lab, Jamshedpur, India) was used to obtain thin coating on orthodontic brackets. The AgO nanoparticle dispersion was created in an appropriate solvent using ethanol, after which the cleaned brackets were immersed in the nanoparticle dispersion solution for 25 minutes. To achieve an even coating, the brackets are pulled out at a controlled speed. The coated brackets are then dried at room temperature or in a low-temperature oven to eliminate any solvent residue. (Figure *4*)

FIGURE 4: Silver oxide nanoparticle coating by dipping the brackets in solution



Characterization of Silver oxide nanoparticles
The morphology and chemical composition of AgONPs were analyzed using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDAX), respectively. Scanning electron microscopy (SEM, Zeiss EVO LS15, Bangalore, India) , SEM characterization showed a uniform coating of 10-nm-thick silver oxide film was coated on orthodontic brackets.(Figure 5-*6*) Elemental analysis of AgONPs was done using EDAX, and the peak values showed that the predominant element in the sample was Silver(Figure 7).

FIGURE 5: SEM Characterization on uncoated bracket
SEM, scanning electron microscopy

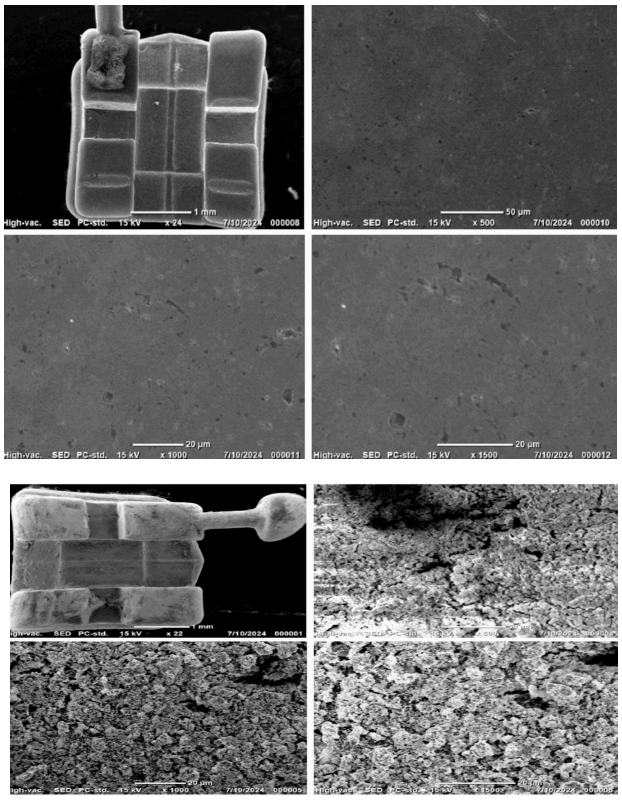


FIGURE 6: SEM Characterization on bracket coated with silver oxide nanoparticles

SEM-scanning electron microscopy

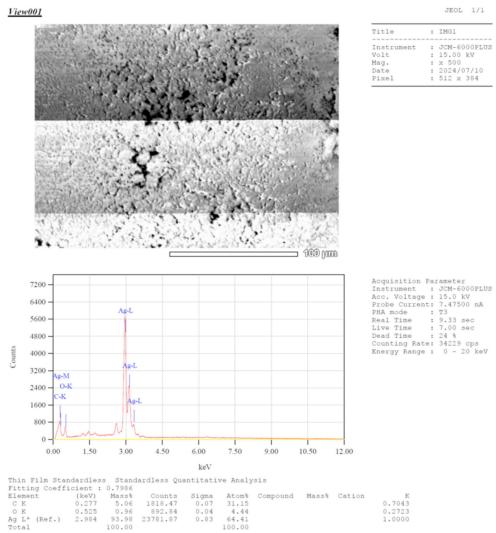


FIGURE 7: Elemental analysis of powdered AgONPs using EDAX

EDAX, energy-dispersive X-ray spectroscopy; AgoNPs, Silver oxide nanoparticles

CULTURE PREPARATION

Luria Bertani (LB) broth (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Distilled water 1000mL) 30mL was prepared in Erlenmeyer's flask and autoclaved at 121°C for 15 minutes. Later, *S. mutans* strain (MTCC 497) was inoculated in 30mL of sterilized LB broth flask and incubated at 37˚ C for 48h. After incubation, Cultured strain was centrifuged at 6000rpm for 10 minutes respectively, supernatant was discarded and the pellets were dissolved in 1% (w/v) Sodium chloride and adjusted to absorbance 1.000 at 600nm under UV spectrophotometer (Genesys 10S UV-VIS Spectrophotometer).

SPECIMEN PREPARATION

The samples containing 10 brackets in each group (A, B and C) were autoclaved at 121˚C for 15mins. Each bracket was placed in a micro centrifuge tube containing 1mL of sterile LB broth and 0.1mL prepared inoculum respectively and incubated at 37˚C for 24h. After incubation, the brackets were immersed in 1mL of 1% (w/v) sodium chloride solution and 0.1mL of bracket suspension was poured in a sterile petri plate respectively and 20mL of LB agar media (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Agar 20g, Distilled water 1000mL) was poured (pour plate method) and allowed to solidify, incubated at 37˚ C for 24h. 24h after incubation CFU was calculated.

Brackets in Group A and Group C were immersed in 0.2% Chlorhexidine mouthwash(CLOHEX) for one minute twice daily for a period of 14 days. After plating the brackets were added back into the respective tubes containing bacterial culture. After a period of 14 days the brackets were reassessed for number of CFU of *S.mutans* using LB agar medium.(Figure *8-12*)

FIGURE 8: Innoculation of *S.mutans* strain

***S.mutans* - Streptococcus mutans**

FIGURE 9: Infecting brackets with *S.mutans* strain

S.mutans- Streptococcus mutans

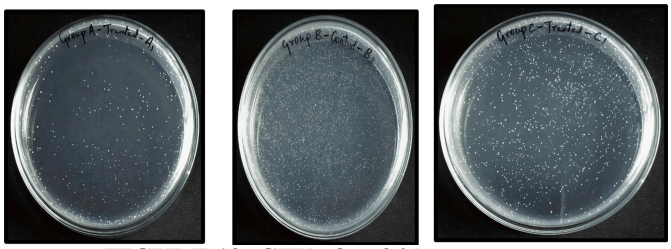


FIGURE 10: CFU after 24 hours

CFU - colony forming units

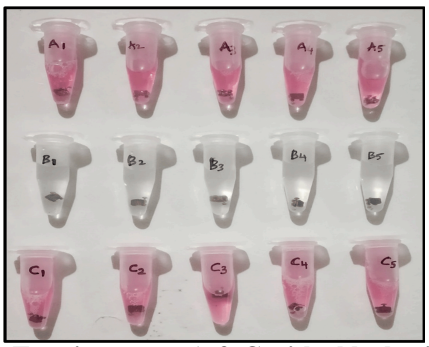


FIGURE 11: Treating group A & C with chlorhexidine mouthwash

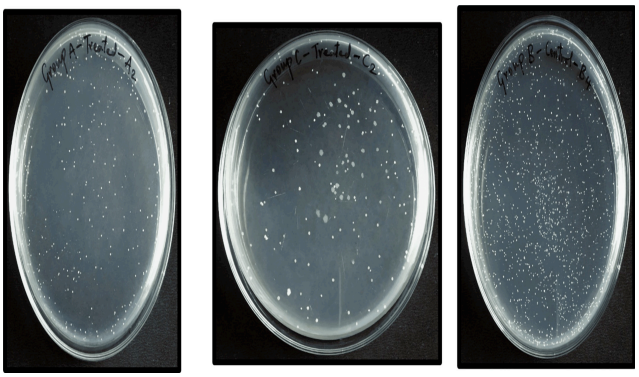


FIGURE 12: CFU after 14 days
CFU - colony forming units

STATISTICAL ANALYSIS:

After tabulating the values, the data were analyzed and compared using Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp. Descriptive analysis includes expression of CFUs of S.mutans in terms of mean and standard deviation. To assess inferential statistics One - way ANOVA test followed by Tukey's post hoc analysis/ Kruskal Wallis Test followed by Dunns post hoc test will be used to compare the CFUs of *S. mutans* between 3 groups. The level of significance [P- Value] will be set at P<0.05.

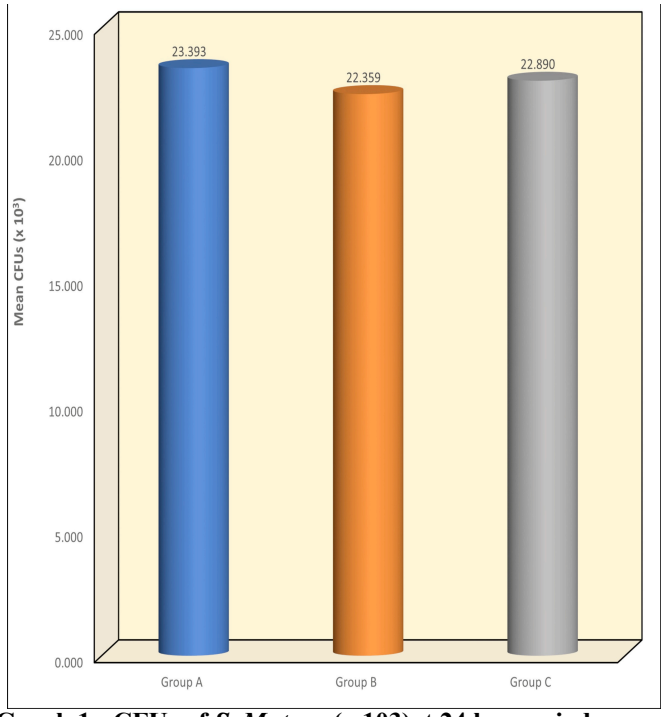
RESULTS

The groups mentioned in the results are as follows:
Group A: Plain orthodontic brackets immersed in 0.2% chlorhexidine mouthwash.
Group B: Orthodontic brackets after coating with AgO nanoparticles.
Group C: Orthodontic brackets after coating with AgO nanoparticles and immersed in 0.2% chlorhexidine mouthwash.
Antimicrobial Analysis After 24 hours
Table no. 1 provides a comparison of the mean Colony Forming Units (CFUs) of *S. Mutans* at a 24-hour period between 3 groups. Group A displayed a mean CFU count of 23.393 ± 1.441, with observed values ranging from 21.64 to 25.82. Group B had a mean CFU count of 22.359 ± 1.079, with a range between 20.48 and 23.52, and Group C showed a mean CFU count of 22.890 ± 1.321, with values spanning from 21.08 to 24.78. Despite the differences in mean CFU counts, the p-value for the comparison among the three groups is 0.22, indicating that these differences are not statistically significant. This suggests that at the 24-hour period, there was no significant variation in the mean CFU counts of *S. Mutans* across the three groups.(Graph 1)

Table no. 1 Comparison of mean CFUs of *S. Mutans* (x 10^3) at 24 hrs period b/w 3 groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	p-value
Group A	10	23.393	1.441	21.64	25.82	0.22
Group B	10	22.359	1.079	20.48	23.52	
Group C	10	22.890	1.321	21.08	24.78	

TABLE 1: The table no. 1 provides a comparison of the mean Colony Forming Units (CFUs) of *S. Mutans* at a 24-hour period between 3 groups.



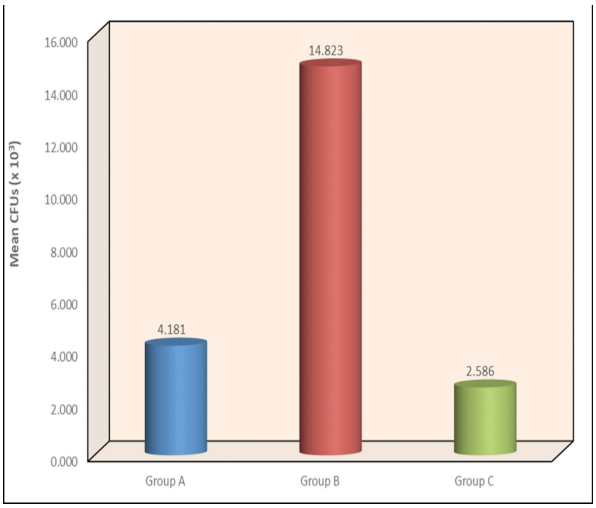
Graph 1 : CFUs of *S. Mutans* (x 103)at 24 hrs period b/w 3 groups

Antimicrobial Analysis after 14 days

Group A exhibited a mean CFU count of 4.181 ± 1.295, with a range from 2.44 to 7.12. On the other hand, Group B had a mean CFU count of 14.823 ± 1.480, with values ranging from 12.59 to 16.64. Group C showed mean CFU count of 2.586 ± 1.243, with values spanning from 1.12 to 4.32.(table *2*)
The results for the comparison between the three groups was p<0.001, indicating a statistically significant difference in the mean CFU counts of *S. Mutans* among the three groups at the 14-day period. (Graph 2)

Table no. 2 Comparison of mean CFUs of S. Mutans ($x 10^3$) at 14 days period b/w 3 groups using One-way ANOVA Test						
Groups	N	Mean	SD	Min	Max	p-value
Group A	10	4.181	1.295	2.44	7.12	
Group B	10	14.823	1.480	12.59	16.64	<0.001*
Group C	10	2.586	1.243	1.12	4.32	

TABLE 2: * - Statistically Significant The table no. 2 presents the comparison of mean Colony Forming Units (CFUs) of *S. Mutans* at a 14-day period among three groups

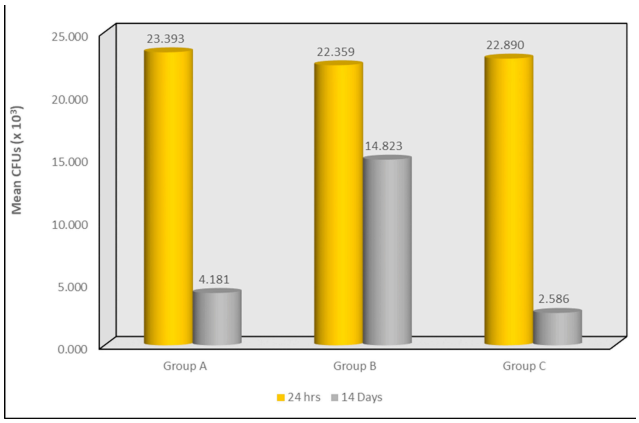


Graph 2 : CFUs of *S. Mutans* (x 103) after 14 days b/w 3 groups

The multiple comparison of mean differences in Colony Forming Units (CFUs) of *S. Mutans* at a 14-day period reveals the following findings among the three groups. Group C showed significantly least mean CFU counts compared to Group A, with mean difference of 1.595 (95% CI, 0.106 to 3.084) and Group B, with mean difference of 12.337 (95% CI, 10.748 to 13.726) and the mean differences were statistically significant at p=0.03 & p<0.001 respectively. This was then followed by Group A which showed significantly lesser mean CFU counts as compared to Group B, with mean difference of -10.642 (95% CI, -12.131 to -9.153) and the mean difference was statistically significant at p<0.001. This implies that, Group C showed significantly least mean CFUs count, followed by Group A and highest in Group B at 14 days Incubation period.(Graph 3) (Table *3*)

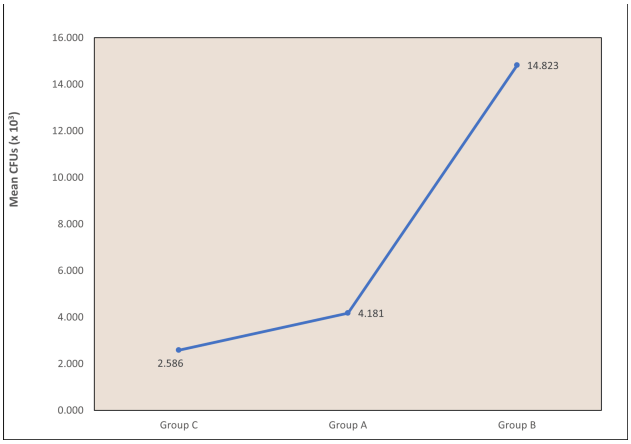
TABLE 3: *-Statistically Significant The multiple comparison of mean differences in Colony Forming Units (CFUs) of *S. Mutans* at a 14-day period reveals the following findings among the three group

Table no. 3 Multiple comparison of mean diff. in CFUs of S. Mutans ($x 10^3$) at 14 days period b/w 3 groups using Tukey's Post hoc Test					
(I) Groups	(J) Groups	Mean Diff.(I-J)	95% CI for the Diff		p-value
			Lower	Upper	
Group A	Group B	-10.642	-12.131	-9.153	<0.001*
	Group C	1.595	0.106	3.084	0.03*
Group B	Group C	12.237	10.748	13.726	<0.001*



Graph 3: **mean CFU of *S.mutans* between 24hrs and 14days period in each group**

The analysis aimed to compare the mean Colony Forming Units (CFUs) of *S. Mutans* at 24 hours and 14 days across three groups, as well as the percentage reduction in CFU counts over the 14-day period. At the 24- hour mark, there were no significant differences in CFU counts among the three groups, with near similar mean values observed. However, at the 14-day period, all three groups showed significant reductions in CFU counts. Group C demonstrated the most substantial reduction, followed by Group A, with Group B showing the least reduction. This trend was also reflected in the percentage reduction of CFUs, with Group C having the highest mean percentage reduction, followed by Group A, and Group B exhibiting the lowest percentage reduction. The analysis highlights that the 14-day incubation period significantly impacted the reduction of CFU counts in all groups, with the most pronounced effect observed in Group C, indicating its effectiveness in reducing the presence of *S. Mutans* over time.(Graph 4)



Graph 4 :CFUs of *S. Mutans* (x 103)at 24 hrs period b/w 3 groups [Arranged in Ascending order]

The study observed significant reductions in Colony Forming Units (CFUs) of *S. Mutans* over a 14-day period across all three groups. The orthodontic brackets coated with AgO Nano particles and immersed in 0.2% chlorhexidine mouthwash, exhibited the greatest reduction in CFU counts. This was followed plain orthodontic brackets immersed in 0.2% chlorhexidine mouthwash, which also showed a substantial reduction. The group with orthodontic brackets after coating with AgO Nano particles, demonstrated the least reduction among the three groups. These findings suggest that the combination of AgO Nano particles coating and chlorhexidine mouthwash in Group C was the most effective in reducing the CFUs of overtime.

DISCUSSION

Orthodontic treatment seeks to achieve an occlusion that is both functionally and esthetically appealing. For the treatment to be successfully completed, it is essential to maintain the health of both hard and soft tissues throughout the entire duration of the procedure. Orthodontic appliances change the physicochemical environment for bacterial growth, leading to both qualitative and quantitative shifts in bacterial populations. Furthermore, orthodontic appliances not only facilitate the build-up and retention of food particles and debris but also may shield plaque from the effects of brushing and chewing. The level of *S. mutans* has shown to be significantly higher after insertion of fixed orthodontic appliances and increases the risk of caries development (Zachrisson, 1974). This could be attributed to the presence of *S. mutans* in areas that retain plaque and the challenges of removing the bacteria through mechanical cleaning methods [14].

Maintaining good oral hygiene can be particularly difficult when bands, wires, and ligatures are in place. Effective mechanical plaque management remains the most direct and efficient approach to disrupting the formation of supragingival biofilm. Studies show that consistent and systematic brushing combined with flossing at regular intervals can help prevent and even reverse inflammatory changes in gum tissues. Furthermore, mouth rinses that contain chemotherapeutic agents can significantly enhance mechanical methods of plaque elimination in both the prevention and treatment of periodontal disease. CHX is regarded as safe, stable because of its high substantivity, effective in preventing and managing plaque formation, disrupting existing plaque, inhibiting the development of gingivitis, and alleviating symptoms of periodontitis [15].

Nanotechnological approaches to combat biofilm are based on the use of nano-particles on biomaterials. Surfaces of these biomaterials are functionalized through impregnating or embedding nano materials on/into the substrate [16]. AgNPs can lead to the loss of bacterial cell membrane integrity and cell wall permeability, particularly the blocking of bacterial adhesion and biofilm formation. There is a growing belief that a combination of antibacterial agents is a promising strategy against oral infections, which may increase biofilm inhibition and reduce the ever-increasing risk of antibacterial resistance [17]. Limited research exists on the synergistic effect of chlorhexidine and silver oxide nanoparticles on antimicrobial activity against *S.mutans* . This study evaluated the antimicrobial efficacy of silver oxide nano particles and chlorhexidine mouthwash when used alone and in combination on orthodontic brackets.

Group C showed a statistically significant reduction in the CFU of *S.mutans* in 24h and after 14days. Similar results were obtained by Meng-meng Lu et al. [17] reported a significantly improved inhibitory effect against Streptococcus mutans biofilms using a dual CHX-silver nanoparticle delivery system. However, a key distinction between our study and meng-meng Lu et al. lies in the mode of antimicrobial delivery. While Lu et al. utilized a nanoparticle-based drug delivery platform capable of redox- and pH-responsive release of CHX and silver ions, the present study evaluated AgO-coated brackets combined with conventional CHX mouthwash . Ahmad Gholami et al [18] , who demonstrated superior antibacterial efficacy of CHX-loaded positively charged silver nanoparticles against Enterococcus faecalis compared to CHX or silver nanoparticles alone . In another study by Seetharam Charannya et al [19] concluded that synergistic effect of CHX-AgNP combined solution exhibited the highest efficacy in comparison to these solutions used alone as endondontic irrigants against Enterococcus faecalis, Klebsiella pneumoniae, and Candida albicans .The combination of silver oxide nanoparticles and chlorhexidine presents a highly promising strategy for combating Streptococcus mutans, a primary bacterium responsible for dental caries. The synergy may be attributed to continuous release of silver ions and long-lasting binding of CHX to tissues. Future research should measure the release profile and mechanism of action to confirm the synergy.

In contrast to the present study, a study conducted Nikita P. Panpaliya et al solution containing silver nanoparticles showed significantly higher bacteriostatic and bactericidal effect against five different oral pathogenic bacteria compared to chlorhexidine [20]. This was also in agreement with studies conducted by Farzaneh Ahrari et al, Alexandros Besinis et al and Raul Alberto Morales Luckie et al which concluded that nanoparticles had superior

antimicrobial activity compared to chlorhexidine mouthwash [21-23]. In our present study chlorhexidine shows better antimicrobial activity when compared to brackets coated with silver oxide nanoparticles this could be because of repeated exposure of the brackets to fresh solution of chlorhexidine mouthwash twice daily for 1 minute and due to the reduction in the bioavailability of nanoparticles over a period of time as it is embedded into the brackets.In the current in vitro study, we tried to integrate nanoscience, chemistry, and bacteriology to obtain a new disinfectant for orthodontic purposes. AgONPs when combined with CHX proved to enhance the antimicrobial effectiveness. However, this method is done under in vitro conditions, and hence the presence of blood, temperature changes, or variations in the oxidation and reduction at different areas of the oral cavity may affect the results. Further studies on the effect of CHX-AgONP on other bacterial species as well as cytocompatibility assessments of CHX-AgONP against human cells are needed to evaluate its application as a new disinfectant adequately.

Clinical Implications

The findings of this study indicate that the combination of silver oxide nanoparticles with 0.2% chlorhexidine provides a superior antimicrobial effect on orthodontic brackets compared to either agent used alone. This enhanced activity significantly minimize the early microbial load around fixed appliances, a period when patients are most vulnerable to enamel demineralization and the development of white spot lesions. Implementing this combination as a simple chairside pretreatment for brackets before bonding could serve as a practical, low-cost preventive strategy to improve oral health outcomes. The findings also support the broader concept of antimicrobial-modified orthodontic biomaterials, which can help minimize plaque retention, reduce gingival inflammation induced by orthodontic appliances.

LIMITATIONS

Within the limitations of this in vitro study, the following conclusions were drawn. However, further in vitro and in vivo investigations on the synergistic effect of chlorhexidine and silver oxide nanoparticles are necessary to obtain clinically relevant data. In vitro studies generally assess antimicrobial effects against selected pathogens, which may not fully represent the complex microbial flora present in the oral cavity; therefore, future studies should include a wider range of microbial species. Additionally, the mechanical and functional properties of nanoparticles observed under in vitro conditions may differ in vivo, indicating the need for further research to thoroughly understand their behavior and their role in enhancing antimicrobial effects in clinical settings . Further studies on the effect of CHX-AgONP on other bacterial species as well as cytocompatibility assessments of CHX-AgONP against human cells are needed to evaluate its application as a new disinfectant adequately.Future research should measure the release profile and mechanism of action to confirm the synergy.

CONCLUSIONS

Within the limitations of the present study, it can be concluded that combination of silver oxide and chlorhexidine (CHX) emerges as the most effective approach. This synergy leverages the broad-spectrum antimicrobial properties of CHX and the sustained release of silver ions from silver oxide, providing enhanced protection against bacterial biofilm formation. The diverse potential of NPs should be judiciously exploited to develop novel medicaments which can combat problems of antimicrobial resistance..

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