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A comparative evaluation of the antimicrobial efficacy of Chlorhexidine and Chlorine dioxide on self-ligating brackets contaminated with *Streptococcus mutans* biofilm- An In vitro study

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ABSTRACT

Objective: To evaluate and compare antimicrobial efficacy of Chlorhexidine and Chlorine dioxide mouthwashes on *S.mutans* biofilm created on metal and ceramic self-ligating brackets.

Materials and methods: A total of 162 metal and ceramic self-ligating brackets (3M™ SmartClip™ & Clarity SL™) were randomly divided into 3 groups and 2 subgroups. Standard procedures were followed to coat all brackets with *S.mutans* biofilm. The biofilms were cultivated which were then subjected to the effects of the mouthwashes. Quantitative assessment was carried out by comparing the number of viable colonies of *S.mutans*. A Mann-Whitney *U* test was used to compare the data between the experimental and control groups. ($p < 0.05$).

Result: When compared to untreated controls the antimicrobial efficacy of Chlorhexidine Digluconate and Chlorine Dioxide mouthwashes was found to be statistically significant ($p = 0.00$). The comparison between Chlorhexidine digluconate and Chlorine dioxide mouthwashes was not statistically significant in Ceramic self-ligating group ($p = 0.502$) and statistically significant in Metal self-ligating group ($p = 0.001$)

Conclusion: *S mutans* colonies on metal and ceramic self-ligating brackets can be reduced effectively by Chlorhexidine digluconate and Chlorine dioxide mouthwashes. Chlorhexidine digluconate more effective for metal bracket group. Both mouthwashes had comparable antimicrobial effectiveness, with the difference in the number of viable colonies following exposure for ceramic bracket groups.

1. Introduction

The fixed orthodontic therapy consists of active and passive components, of which brackets play a pivotal role throughout the orthodontic therapy. Fixed orthodontic appliances poses a **substantial challenge to meticulous oral hygiene routines**, facilitating the accumulation and stagnation of plaque around brackets and wires. Areas of demineralized enamel due to orthodontic therapy i.e. white spot lesions having prevalence of 2 %–96 %, are typically the result of accumulated plaque buildup.^{1,2} Fixed appliances disrupt the natural self-cleansing mechanisms of the oral cavity, primarily due to variations in surfaces of brackets, bands, and wires creating shelter that harbors microorganisms, impeding the natural microbial balance within

established oral ecosystems. These can instigate a cascade of infections and cross-contamination.³ *S.mutans* plays an important role in this cariogenic process leading to the formation of white spot lesions which are commonly seen in patients undergoing orthodontic treatments. *S. mutans* is also the most prevalent biofilm-associated bacteria.⁴

Advancement in orthodontics have led to the development of a multitude of bracket designs and appliances which can lead to better oral care maintenance by orthodontic patients.^{5,6} Despite these advancements, the problem of biofilm development on brackets still persists in orthodontic practice.⁷ Evidence suggests that the bracket material has an undeniable impact on bacterial adherence and plaque accumulation but demonstrated that surface roughness, surface free energy, and other physicochemical properties of biomaterials

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significantly influence their capacity to retain dental plaque.^{8,9} The adhesion of biofilm, a key indicator of potential oral health complications, was observed to be considerably reduced on metal brackets relative to their ceramic counterparts.¹⁰

The scientific literature describes a multitude of antimicrobial options exist, with varying chemical origins, mechanisms, and clinical presentations.¹¹ Chlorhexidine digluconate (CHX) stands as the preferred antimicrobial agent due to its broad-spectrum bactericidal activity against both gram-positive and gram-negative bacteria, despite variations in optimal exposure time.^{12,13} While CHX boasts potent antimicrobial properties, its use is not without potential side effects including altered taste perception, dental and mucosal discoloration, and, in rare cases, mucosal shedding and parotid gland swelling. Among these visible side effects, primarily brownish discoloration of teeth and tongue surface.^{12,13} Notably, patients find the taste of CIO2 more palatable than CHX, and unlike the latter, it lacks evidence of long-term effects like staining. Investigations revealed the multifaceted antimicrobial capabilities of CIO2, encompassing both bactericidal and virucidal efficacy, highlighting its potential as a broad-spectrum disinfectant.¹⁴

In light of ongoing concerns regarding the CHX and the promise of

CIO2, this research endeavored to evaluate their comparative antibacterial efficacy against *S.mutans* biofilms on ceramic and metallic self-ligating brackets, with a view to identifying a viable alternative for routine use in patients undergoing fixed orthodontics.

2. Materials and methods

The present study was conducted in the Department of Orthodontics Manav Rachna Dental College Faridabad. Institutional ethical clearance (MRDC/IEC/2020/03) was taken before commencing the study. Sample size estimation using GPower software (version 3.0). The sample size was estimated from a study by Aithal et al. 2019³ for F tests - ANOVA: Fixed effects, special, main effects, and interactions were chosen. A minimum total sample size of 158 was sufficient for an alpha of .05, a power of 80 %, and an effect size of .25. The sample was rounded off to 162 to ensure an equal number of samples in all subgroups. The sample of 162 brackets was divided into 3 groups and 2 subgroups, i.e., Group 1- Control group, Group 2- CHX, and Group 3- CIO2 group consisting of 54 brackets in each group. This was subdivided randomly into 2 subgroups i.e., ceramic and metal bracket groups (27 each) (Fig. 1).

S. mutans bacteria was acquired from the microbiology lab. *S. mutans*

CONSORT FLOW DIAGRAM

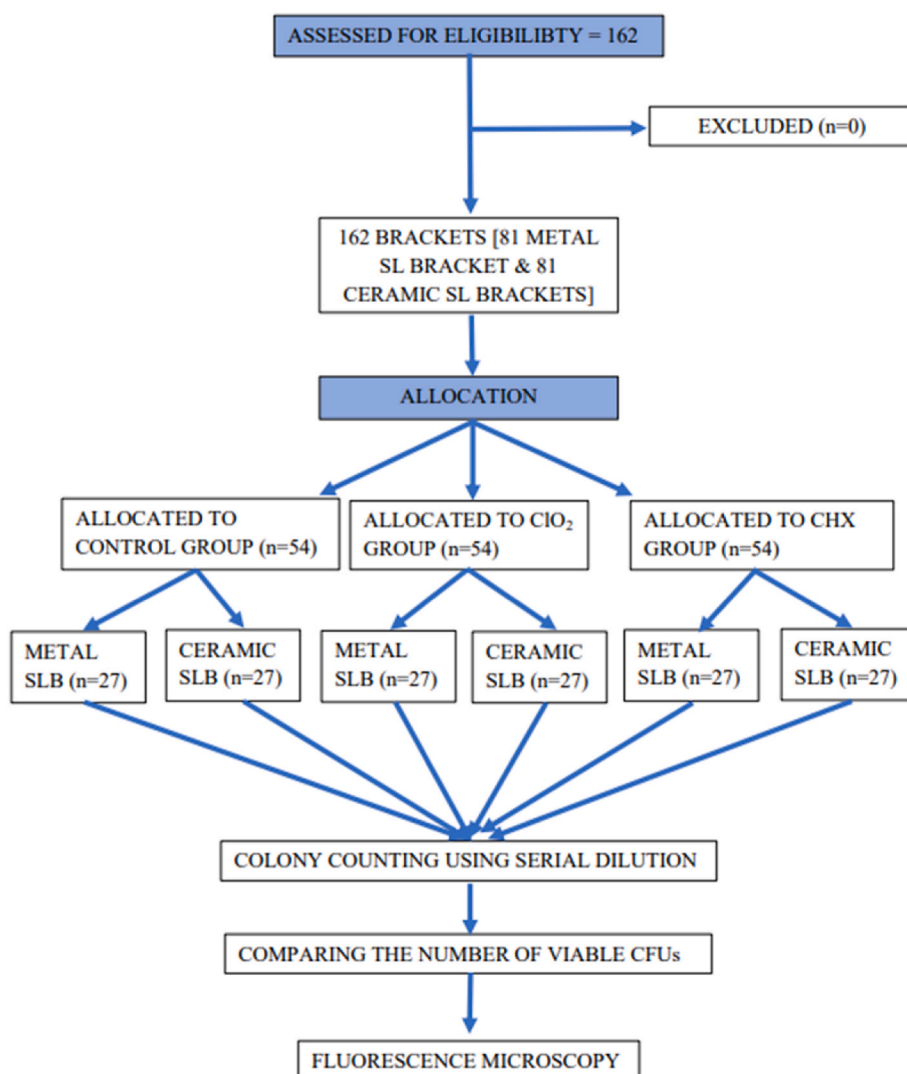


Fig. 1. CONSORT chart.

(Strain type 10449) was inoculated in BHI broth (brain heart infusion) + 1 % glucose solution. Under microaerophilic conditions, this was incubated for 18 h at 37 °C (5 % CO₂). After centrifuging the bacterial culture for 5 min at 3000 RPM, the supernatant was discarded. After that, the cell pellet was resuspended in 5 mL of sterile .9 percent Phosphate buffer (PBS).

Brackets were autoclaved and verified to be sterile using *GKE Steri-Record*® self adhesive indicator according to EN ISO 11140-1 type 5 indicators. In a 24-well plate, sterilized brackets were incubated with 1000 mL microorganism suspension (brain heart infusion with 1 % glucose). For biofilm formation, the plates were incubated anaerobically at 37 °C as a static culture for 24 h. Brackets were then agitated to remove biofilm from the bracket surface so that colony-forming units (CFU) could be counted and for convenient counting of the CFUs/ml of suspension was calculated and converted to logarithm (log₁₀).

CHX (Hexidine™, ICPA health products Ltd. - [.2 % w/v]) and Chlorine dioxide (Freshclor™, Group Pharmaceuticals Ltd. - [.2 % w/v]) mouthwashes were used on the *S mutans* biofilm on self-ligating brackets (3M™ SmartClip™ SL3 Self-Ligating Brackets). The contaminated brackets from the 4 groups (Metal SL – CHX, Metal SL – ClO₂, Ceramic SL – CHX, Ceramic SL – ClO₂) were immersed in the treatment solutions for 1 min each as per recommendations of manufacturers, except for the rest of the 2 untreated groups with mouthwashes (metal and ceramic control groups).

The brackets were diluted immediately after being treated with CHX and ClO₂ mouthwashes in a 5 % phosphate buffer solution (PBS) for analysis after. The data were collected before and after the effects of both mouthwashes.

3. Statistical analysis

Data were entered into Microsoft Excel spreadsheet (version 10) and checked for any missing entries. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21. The normality of the data was checked by Shapiro-Wilk test and Kolmogorov-Smirnov test (Table 1). Both the tests showed that the data did not adhere to normality, therefore, non-parametric tests were used for comparison. A Mann-Whitney U test was applied to compare the differences among all the studied groups. The level of significance was set at *P* < 0.05.

4. Results

The mean viable colonies in metal self-ligating bracket biofilms after submerging in CHX (.56) is lower than those of ClO₂ (2.81) with the highest number of colonies being 3 in CHX whereas 8 in ClO₂ (Table 2).

The mean viable colonies in ceramic self-ligating bracket biofilms after being subjected to antimicrobial effects of CHX (1.22) is lower than those of ClO₂ (1.41) with the highest number of colonies being 3 in CHX whereas 8 in ClO₂. This group revealed that ClO₂ is more effective on ceramic brackets (1.41) than on metal brackets (2.81) whereas the effect of CHX is better on metal brackets (.56) than on ceramic brackets (1.22). (Table 2) (Figs. 2 and 3).

When the CFU was checked in ceramic bracket groups, there was no

Table 1
Test for normality.

Group		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	P value	Statistic	df	P value
CHX	Metal	.349	27	.000	.711	27	.000
	Digluconate	.229	27	.001	.784	27	.000
Chlorine Dioxide	Metal	.171	27	.041	.896	27	.011
	Ceramic	.251	27	.000	.841	27	.001
3.00	Metal	.106	27	.200*	.941	27	.129
	Ceramic	.158	27	.082	.964	27	.460

Table 2
Intragroup Comparison of effectiveness with Ceramic brackets and Metal Brackets.

Group	Group	Mean	SD	Mean Ranks	P value
Metal	CHX Digluconate	.56	.80	20.43	.0001 ^a
	Chlorine Dioxide	2.81	2.45	34.57	
	Control	62.19	23.67	68.00	
Ceramic	CHX Digluconate	1.22	1.55	26.13	.0001 ^a
	Chlorine Dioxide	1.41	1.39	28.87	
	Control	66.56	20.33	68.00	

^a Significant.

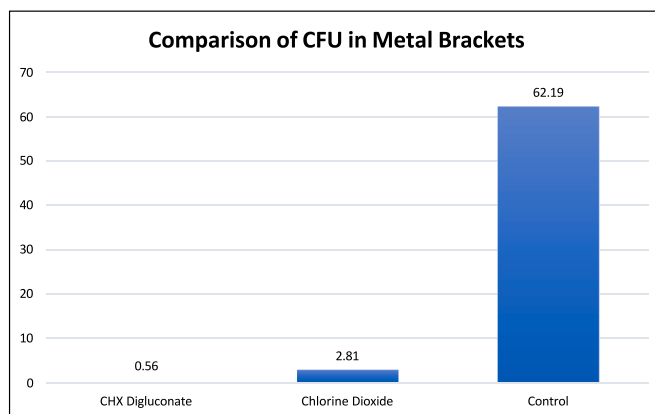


Fig. 2. Intragroup comparison of colony forming units with metal brackets.

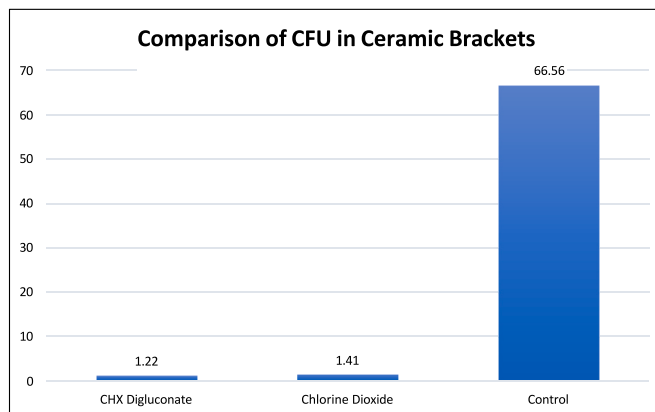


Fig. 3. Intragroup comparison of colony forming units with Ceramic brackets.

significant difference in CFU count between the two groups with *P* = 0.502. There was a significant difference in CFU count between the two groups in metal bracket groups alone, with the *S.mutans* CFU count being significantly high in the ClO₂ rinse group with (*p* = 0.001). (Table 2). However, when compared to the untreated controls, both groups showed clinically significant reduction in bacterial colonies (*p* = 0.00).

5. Discussion

In search of finding a new mouthwash as compared to CHX with less side effects, the present study was designed to compare and evaluate the antimicrobial efficacy of CHX and ClO₂ mouthwashes.

Our results concluded the metal self-ligating bracket CFU count after being treated with CHX is significantly lower than those of ClO₂ (*p*=0.001), therefore CHX is more effective than ClO₂ for the metal brackets. It can be attributed to the nature of the two types of

mouthwash, CHX works by disrupting the cell membranes and coagulating cytoplasmic proteins whereas CIO2 mouthwash has antimicrobial activity principally caused by the oxidative destruction of essential macromolecules.

However, for the ceramic self-ligating bracket biofilm CFU count after being subjected to antimicrobial effects of CHX is lower than those of CIO2 numerically but non significantly i.e. ($p = 0.502$), both have similar efficacy against *S. mutans*. We attribute this to the difference in the surface topography of polycrystalline alumina oxide ceramic brackets and metal brackets. Ceramic brackets being the more porous of the two allow higher bacterial biofilm adhesion. The antibacterial property of CHX is similar that of CIO2 on some important oral pathogens i.e. *S. mutans*, *L. acidophilus*, *E. faecalis*, *V. alcalescens*, *E. corrodens*, *A. actinomycetemcomitans* and *C. albicans* so it supports the outcome of this study.

When compared to untreated controls, the results revealed that the antibacterial efficacy of CIO2 and CHX mouthwashes is statistically significant ($p = 0.00$). Therefore, although CHX mouthwash is considered to be the gold standard, CIO2 is also effective for *S. mutans* biofilm control.¹⁵ In contrast to the work of Paraskevas and coworkers who concluded that CIO2 was less effective than CHX based on the alteration in plaque index PI after a very short period of time, only 3 days of use, this study found similar efficacy of CHX and CIO2 for ceramic brackets.^{14,20}

In contrast to non-orthodontic patients, orthodontic patients typically struggle to maintain appropriate oral hygiene, which results in a build-up of biofilms and qualitative alterations in the local flora.^{16–18} We chose self ligating brackets (SLBs) for our study as the design of the SLBs and the absence of wire or elastomeric ligatures, according to some studies, make them more hygienic and less prone to bacterial colonization.⁸ The SLBs do not have an advantage over conventional brackets with respect to periodontal status and halitosis. So, this is subject to controversy.¹⁹

To eliminate this ongoing debate, we have used metal SLBs as well as ceramic SLBs in our study. The present study has various limitations, such as the study was carried out under laboratory settings which could not replicate absolute oral environment. The ceramic SLBs used in the study had a metallic clip meant for the active wire engagement. Hence, were not purely ceramic. Further in vivo investigations are required to support these findings and RCTs can be done to conclude the strong evidence in support to the present findings.

6. Conclusion

The outcomes of the study are as follows-

- The control group has significantly higher *S. mutans* CFU.
- The findings of this study concluded that CIO2 has similar antimicrobial efficacy against *S. mutans* as that of CHX with ceramic SLBs.
- The CHX is more effective as compared to CIO2 with metal SLBs.

No conflict of interest

The undersigned author transfers all copyright ownership of the manuscript (A COMPARATIVE EVALUATION OF THE ANTIMICROBIAL EFFICACY OF CHLORHEXIDINE AND CHLORINE DIOXIDE ON SELF-LIGATING BRACKETS CONTAMINATED WITH STREPTOCOCCUS MUTANS BIOFILM) to Journal of oral biology and craniofacial research in the event the work is published. The undersigned author warrants that the article is original, is not under consideration for publication by another journal and has not been previously published. I sign for and accept responsibility for releasing this material on behalf of any and all co-authors.

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proofs.

By the Corresponding Author on behalf of the all-other authors.

Patient's/Guardian's consent

Not Applicable, as this is an in vitro study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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