Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [8] July 2023 : 213-218

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Comparative Evaluation of Microbial Contamination of Orthodontic Microimplants and Assessment of The Disinfecting Efficiency of Chlorhexidine, Isopropyl Alcohol and UV Light On Them - An *In Vitro* Study

Sunil Kumar Nagmode1, Manik Saswadkar2, Hrushikesh Aphale3, Vivek Shinde4, Mrunal Adbhai2, Sejal Surana2, Akshay Chaugule2, Akshay Bhole2
1HOD and Professor, Department of Orthodontics and Dentofacial Orthopedics, SMBT Dental Collage and Hospital, Sangamner
2Post graduate student, Department of Orthodontics and Dentofacial Orthopedics, S.M.B.T Dental College and Hospital, Sangamner
3 Professor, Department of Orthodontics and Dentofacial Orthopedics, SMBT Dental Collage and Hospital Sangamner
4 Reader, Department of Orthodontics and Dentofacial Orthopedics, SMBT Dental Collage and Hospital Sangamner

ABSTRACT
Bacterial contamination of TAD's can have significant effect on patient oral health. Inflammation of tissue surrounding TADs produces an increase in orthodontic miniimplant failure by 30 %. So to study microbial contamination of orthodontic microimplants and to assess the disinfective efficiency of Chlorhexidine, Isopropyl Alcohol and UV light, this study was carried out. 18 orthodontic implants (Dentos Company) was used in study.This study was performed in two parts. Group I – 9 Orthodontic microimplants (Assessment of microbial contamination of orthodontic microimplants),Group II –9 Orthodontic microimplants (Assessment of disinfective efficiency) .Data was managed using Microsoft Excel. Statistical analysis was carried out by using ANOVA and Tukey's post hoc test . In this study microbial contamination was found. Bacterial colonies of staphylococci and Streptococci was observed on orthodontic microimplants. Clinician should take proper measures of sterilization and disinfection any surgical procedures. UV light was found more effective in sterilization as compared to 0.2 % CHX and 70 % IPA. Further study will be needed to differentiate bacterial colonies.
Key words: disinfection, microimplant, microbial

Received 10 .05.2023 Revised 02.06.2023 Accepted 17.07.2023

INTRODUCTION

The orthodontic tooth movement is carried out by using Orthodontic brackets and arch wires. The orthodontic microimplants is a device that is fixed into bone in short term for enhancing orthodontic anchorage.(1) Microimplants have become very popular in orthodontic community in recent years as skeletal anchorage devices. They are excellent alternatives to conventional orthodontic anchorage systems such as intra-oral dental anchoring units and extraoral headgear devices. (2) Several in vitro and in vivo study studies reported microbial contamination in orthodontic appliances received directly from the manufacturer. (3) Infection in oral cavity can occurs due to use of contaminated instruments or straight use of orthodontic instruments received from manufacturer's packaging without disinfection.

Bacterial contamination of TAD's (Temporary Skeletal Anchorage Device) can have significant effect on patient oral health. Inflammation of tissue surrounding TADs (Temporary Skeletal Anchorage Device) produces an increase in orthodontic miniimplant failure by 30 %.(4) Many studies shows incidence of bacteremia following dental procedures. Many case reports show associating dental procedures or disease with onset of endocarditis. Infective endocarditis is a bacterial infection of heart valves or endothelium of heart. Recent study reported that distribution of Streptococci causing infective endocarditis.. (5) Bacterias like *Staphyloccocus aureus* can causes angular cheilitis, endodontic infections, osteomyelitis of jaws, parotitis.. (6) Hence clinician should reduce bacterial contamination by using different sterilization and disinfection methods.

Chlorhexidine is most favourable disinfectant due to its broad-spectrum bactericidal action against both gram positive and gram-negative bacteria. (7). Chlorhexidine shows reduced bacterial count. 0.2 % Chlorhexidine is commonly used as a mouthwash, shows reduced bacterial colonies in fixed orthodontic

users. (8) Alcohols are effective at eliminating vegetative bacteria and viruses from surfaces. The antimicrobial effectiveness of alcohols is through damage to bacterial cell membrane and subsequent denaturation of cellular proteins. A more effective alcohol is isopropyl alcohol (IPL), which is a fast acting and possesses a broad -spectrum antimicrobial activity. (9) UV chambers are use for sterilization procedures in dental clinics. (10) The objective of study is to evaluate microbial contamination of orthodontic microimplants and assessing the disinfecting efficiency of chlorhexidine, isopropyl alcohol and UV light on them.

MATERIAL AND METHODS

It was invitro study. The study was carried out in Aarmaan Micro Lab, Nashik. Total sample size was 18. 18 Orthodontic microimplants of Dentos company were used. The sampling technique selected is convenience sampling technique. Orthodontic microimplants were immersed individually in test tube. Each container contained sterilized Brain Heart Infusion broth (BHI). Then this test tubes were placed in a incubator for 48 hours at 35 0 C to evaluate bacterial growth. Bacterial growth was assessed based on the change in colour and turbidity of medium in test tube. Test tube that were positive for bacterial growth were subjected to biochemical analysis. Biochemical analysis was performed for tubes that exhibited bacterial growth. Organisms were grown on Agar medium by streak plate technique. Incubation was done at temperature – 35 0 c Time – 48 hours. Plates displaying growth of colonies were subjected to gram staining protocol. Then colonies were observed under microscope to differentiate between gram positive and gram negative bacteria. Based on morphological characteristics of bacteria in each sample, they subjected to biochemical tests for identification. Different tests were performed to identify gram positive and gram negative isolates that involved in contamination of orthodontic preformed bands and elastomeric modules. Bacterial count was determined in colony forming units. Orthodontic microimplants were sterilized in surgical grade paper in Autoclave at 1210C temperature for 15 min to confirm absence of bacterial growth. These orthodontic microimplants were contaminated with Staphylococcus aureus. Then this orthodontic microimplants were grouped in 3 subgroups.

Group I – 3 (0.2 % chlorhexidine) - orthodontic microimplants placed in 0.2 % CHLORHEXIDINE solution in test tube for 5 min, Group II - 3 (70 % isopropyl alcohol) - Orthodontic microimplants placed in 70 % isopropyl alcohol solution for 2 mins,Group III - 3 (UV Chamber) – Orthodontic microimplants placed in UV chamber for 10 min. Disinfective efficiency of 0.2 % CHLORHEXIDINE, 70 % ISOPROPYL ALCOHOL and UV light was determined. Microbiological tests were performed again on orthodontic microimplants to assess the disinfective efficiency.

Data Management and Statistical Analysis –

Data was managed by using Microsoft Excel. Statistical analysis was carried out by using ANOVA and Tukey's post hoc test . ANOVA 'F' test was done to check statistical difference between two or more groups. Followed by Tukey's post hoc test.Tukey's post hoc test to find difference between variables.

RESULTS

In study microbial contamination has been found. Gram positive bacterial colonies were found. Gram negative and fungal infection was absent. UV light was found most effective in disinfection of implants. On intergroup comparative statistics between three study groups using One-way Anova F test, it was observed that lowest microbial contamination was observed in Group C (UVC Light) followed by Group B (70% IPA) and highest contamination was observed in Group A (0.2% CHX). Overall statistically significant difference ($p<0.05$) was observed between three study groups. (Table 1, Graph 1) On pairwise comparative statistics of antibacterial efficacy of three study group in terms of colony forming unit using Tukey's post hoc test, efficacy of Group A (0.2% CHX) was lower than Group B (70% IPA) but the difference was not found to be of statistical significance ($p>0.05$). Group C (UVC Light) had statistical significantly higher ($p<0.05$) efficacy as compared to Group A (0.2% CHX). Group C (UVC Light) had statistical significantly higher ($p<0.05$) efficacy as compared to Group A (0.2% CHX) (Table 2)

DISCUSSION

This invitro study was performed by using 18 microimplants. Initially this implants were placed in test tube.Bacterial growth was assessed by using BHI broth and by incubator incubator for 48 hours at 35 0 C to evaluate bacterial growth.Those showing bacterial growth were subjected for gram staining protocol. Bacterial colonies were determined in colony forming units(CFU).Then colonies will be observed under microscope to differentiate between gram positive and gram negative bacteria. Bacterial count was determined in Colony Forming Units by digital colony counter. Then disinfective efficiency of Chlorhexidine, Isopropyl alcohol and UV light was determined. Orthodontic microimplants was sterilized

in surgical grade paper in Autoclave at 1210 C temperature for 15 min to confirm absence of bacterial growth. These orthodontic microimplants contaminated with Staphylococcus aureus which was grown by using Tryptic soy agar. Then Disinfective efficiency of 0.2% CHLORHEXIDINE, 70 % ISOPROPYL ALCOHOL and UV light was determined. Group C (UV light) was significantly best as compared to Group A (0.2% Chlorhexidine) and Group B (70 % IPA).Group A and Group B does not have any significant difference.

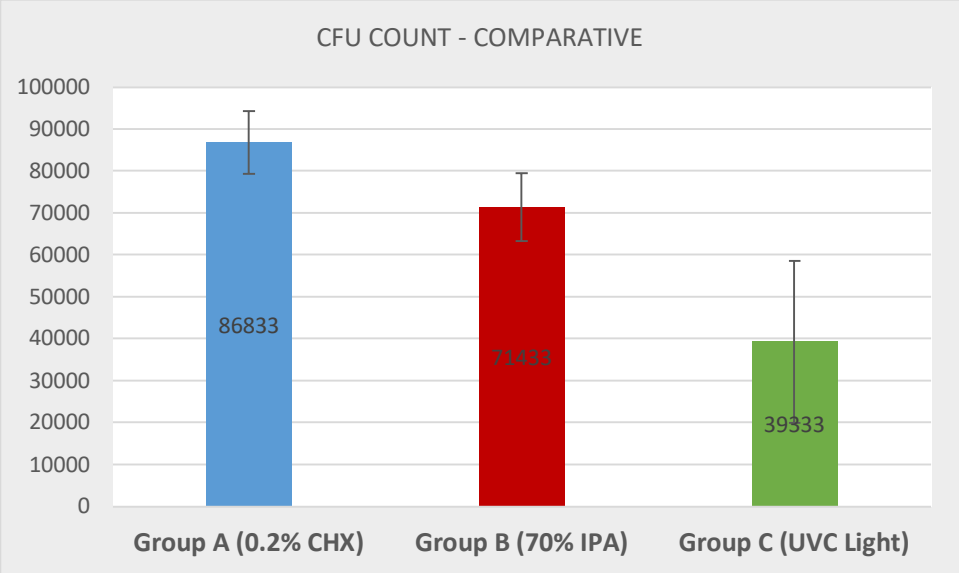
Studies have suggested the need for sterilization or disinfection of materials prior to their administration in the oral cavity[11,12] However, the use of orthodontic appliances directly from the manufacturer's packages is still a routine clinical practice.1According to previous studies, orthodontic appliances received from the manufacturer's packages were unsterile.[13,14,15] Therefore, the present study evaluated the bacterial load of the orthodontic brackets received from different manufacturers and determined the efficacy of chlorhexidine in destroying the microbial contamination. Bacterial colonization was confirmed in all the orthodontic materials received from different manufacturers. The outcome of the current investigation was similar to prior studies using different orthodontic appliances, such as arch wires[13], orthodontic pliers,[16] brackets[15] orthodontic buccal tubes[17] and tooth brushes[18] received from different manufacturers.[13,19,20]These studies indicate that orthodontic appliances used in dentistry are often contaminated with bacteria. In our study, Staphylococci were the predominant organisms isolated from orthodontic brackets. Contamination with Staphylococci mostly occurs due to skin contact during manufacturing and/or packaging of orthodontic appliances.

Similar studies conducted in this regard reported that Staphylococci were the common organisms to contaminate the orthodontic brackets. In previous study on orthodontic brackets, B. cereus and B. licheniformis were the other frequently isolated organisms from the orthodontic brackets, followed by Streptococci [13]. Bacillus spp. cause food-borne diseases as well as nosocomial outbreaks in immune-suppressed hospitalized patients. K. pneumoniae is the respiratory pathogen that was isolated from orthodontic brackets in previous study. The infection spreads from one person to the other through contaminated hands of individuals in the hospital. A similar study conducted by Rastogi et al. isolated Klebsiella spp. from the orthodontic brackets.[14] Further, literature reported a direct association of Klebsiella spp. with autoimmune disorders, such as ankylosing spondylitis, rheumatoid arthritis, and Crohn's disease . Isolation of Lactobacilli spp. that initiate and progress dental caries/decay was relatively low in previous study.[21,22]. All these potential microorganisms are of major health concern; therefore, it is essential to sterilize or disinfect the implants before fixing in the oral cavity. Chlorhexidine used in various medical fields, such as gynecology, urology, and ophthalmology, has a broad antimicrobial activity.[23] Several studies demonstrated that chlorhexidine is effective both as an antiplaque and antimicrobial agent. Depending on different concentrations, it has both bacteriostatic and bactericidal properties.[23,24]Research has further reported that chlorhexidine does not affect the shear bond strength of orthodontic brackets and clinically exhibits acceptable bond strength.[24]Speer et al. also reported that chlorhexidine did not affect the bond strength of metal brackets; however, it reduced the bond strength of ceramic brackets [25]. In this study, two concentrations of 0.2 % chlorhexidine were used to disinfect the orthodontic microimplants received from different manufacturers.

Table 1: Overall intergroup comparative statistics between three study groups using One-way Anova F test

	Mean	SD	One-way Anova F test	p value
Group A (0.2% CHX)	**86833.0**	**7421.8**	**F =10.705**	**p= 0.01***
Group B (70% IPA)	**71433.0**	**8145.0**		
Group C (UVC Light)	**39333.0**	**19295.0**		

***p< 0.05 – significant difference**



Graph 1: Comparative statistics of antibacterial efficacy of three study group in terms of colony forming unit

Table 2: Pairwise comparative statistics of antibacterial efficacy of three study group in terms of colony forming unit using Tukey's post hoc test

Tukey's post hoc test to find pairwise comparison			
Group	**Comparison Group**	**Mean Difference**	**p value, Significance**
Group A (0.2% CHX) vs	**Group B (70% IPA)**	**15400.0**	**p =0.368**
	Group C (UVC Light)	**47500.0**	**p =0.009***
Group B (70% IPA) vs	**Group C (UVC Light)**	**32100.0**	**p = 0.049***

p>0.05 – no statistical significant difference ***p< 0.05 – significant difference**

The exact mechanism exerted by chlorhexidine in destroying the bacteria is not yet clear. [26] However, it has been postulated that positively charged chlorhexidine molecules bind to the negatively charged lipid molecules of the cell membrane and interfere with the process of osmosis [26]. The other novel approach that can be used to reduce the bacterial contamination of orthodontic brackets is application of antimicrobial nanoparticles [27]. The different methods include coating of orthodontic brackets with a thin film of nitrogen-doped titania nanoparticles; combination of glass ionomer or resin-modified glass ionomer cements with fluorapatite, fluorohydroxyapatite, or hydroxyapatite nanoparticles; addition of titania, silica, or silver nanoparticles to acrylic orthodontic materials; and incorporation of nanofillers or silica/titania nanoparticles into orthodontic adhesives.[27]Studies have demonstrated that slightly higher concentrations of chlorhexidine are required to kill gram-negative pathogens than those required to kill the gram-positive pathogens [28,29.] Due to the presence of a permeable cell wall in the gram-positive bacteria, they are destroyed easily when compared to the gram-negative bacteria. Organisms present in were gram-positive bacteria [30]. Therefore, a lower concentration of 0.2 % chlorohexidine was adequate to destroy all the bacteria.

Although unique, the current study has some potential limitations. As the study was conducted in in vitro conditions, further *in vivo* studies are required to support these findings. While orthodontic microimplants showed complete decontamination after treatment with 2% chlorhexidine, there is no data related to long-term effectiveness of chlorhexidine to impede the growth of microorganisms. Overall, the results advocate that the orthodontic implants received from the manufacturer require suitable disinfection to safeguard the patients' health. Furthermore, clinicians should be cautious about the use of contaminated appliances prior to administering in the oral cavity as it might affect the systemic health of the patients.

CONCLUSION

In present study microbial colonies were found on implants. Then colonies will be observed under microscope to differentiate between gram positive and gram-negative bacteria. Bacterial count was determined in Colony Forming Units by digital colony counter. Then disinfective efficiency of Chlorhexidine, Isopropyl alcohol and UV light was determined. Orthodontic microimplants was sterilized in surgical grade paper in Autoclave at 1210 C temperature for 15 min to confirm absence of bacterial growth. These orthodontic microimplants contaminated with Staphylococcus aureus which was grown by using Tryptic soy agar. Then disincentive efficiency of 0.2% CHLORHEXIDINE, 70 % ISOPROPYL ALCOHOL and UV light was determined. Group C (UV light) was significantly best as compared to Group A (0.2% Chlorhexidine) and Group B (70 % IPA).Group A and Group B does not have any significant difference. Clinician should take proper measures of sterilization and disinfection any surgical procedures. UV light was found more effective in sterilization as compared to 0.2 % CHX and 70 % IPA. Further study will be needed to differentiate bacterial colonies.

REFERENCES

1. Paula Argentina Jiman et al, (2021). New and Recovered Temporary Anchorage Devices, In Vitro Assessment of Structural and Surface Properties. Article of Materials. MDPI, 21-26.
2. Mervyn Y. H. Chin et al, (2006). Biofilm formation on surface characterized microimplants for skeletal anchorage in orthodontics. 8900904.
3. Musassique et al,. (2018). Evaluating the sterility of orthodontic materials as received from the manufacturer and that exposed to clinic environment – in vitro study. South Eur J Orthod Dentofac Res 18-24.
4. Xaojing Li, Kristin M Kolltveit, (2000). Systemic Diseases caused by Oral Infection, Clinical Microbiology Review,Vol -13, No-4.100-108
5. M. G. McCormack BDS et al, (2015). Staphylococcus aureus and oral cavity : An overlooked source of carriage and infection ?American Journal Of Infection Control 43; 35-7.
6. P.R Vivek Aithal et al, (2019). In vitro evaluation of microbial contamination and the disinfecting efficacy of chlorhexidine on orthodontic brackets. Progress in Orthodontics, 1;20(1):17.
7. Nioosha Yarahmadi, Farhad Hashemian , Reza Hosseini Doust, (2020). Clinical Effects of Chlorhexidine 0.2 % and Cetylpyridium 0.05 % Combination in comparison with Chlorhexidine, Cetylpyridium and Persica in Reducing Oral Bacteria in Healthy Individuals, Journal of Pharmaceutical Care,30-09.34-39.
8. Tim Sandle, (2017). Disinfection and Decontamination, The Dentist, Page no -34.
9. Rahul Naik, (2016). Assessing of efficacy of ultraviolet chamber in disinfecting dental instruments. Asian Journal of Pharmaceutical and Health Sciences, page no – 1586, 17.
10. Dominique M. Missiakasand Olaf Schneewind (2018). Growth and Laboratory Maintenance of Staphylococcus aureusCurr. Protoc Microbiol 2013 February: chapter 9 : Unit -9C.1, 1.
11. Ganavadiya R, Shekar BC, Saxena V, Tomar P, Gupta R, Khandelwal G. (2014). Disinfecting efficacy of three chemical disinfectants on contaminated diagnostic instruments: a randomized trial. J Basic Clin Pharm. 5:98.
12. Aksoy A, Kılıç G, Hussein E, Aboukhalil D. (2011). Sterilization and disinfection in orthodontics. Principles in contemporary orthodontics: InTech; 20-26.
13. Saad HS, Nidhal GH, A-GB H. Evaluation of microbial contamination of different orthodontic as received arch wires from manufacturers. Int J Med Res Health Sci. 2017;6:13–8.
14. Rastogi S. Assessment of microbial contamination if "as received" and "bench-top exposed" orthodontic materials: A vitro microbiologic investigation. Biomed J Sci Tech Res. 2017;1:729–33.
15. Andrucioli MCD, Nelson-Filho P, Matsumoto MAN, Saraiva MCP, Feres M, De Figueiredo LC, et al. Molecular detection of in-vivo microbial contamination of metallic orthodontic brackets by checkerboard DNA-DNA hybridization. Am J Orthod Dentofac Orthop. 2012;141:24–9.
16. Azeredo F, Menezes LM, Silva RM, Rizzatto SM, Garcia GG, Revers K. Microbiological analysis of orthodontic pliers. Dental Press J Orthod. 2011;16:103–12.
17. Purmal K, Chin S, Pinto J, Yin W-F, Chan K-G. Microbial contamination of orthodontic buccal tubes from manufacturers. Int J Mol Sci. 2010;11:3349–56.
18. Bhat PK, Komalraj M, Alle RS, RK S. Assessment of microbial contamination of the tooth brush head used on orthodontic appliances-a randomized control study. Int J Pharm Sci Invent. 2016;5:18–24.
19. Kharod N, Hemanth M, Kabbur K, Sharmada B, Darsan J. Assessing microbial contamination of as received orthodontic brackets and wires-an in vitro study. Int J Adv Res. 2017;6:1572–5.
20. Dos Santos Gerzson DR, Simon D, dos Anjos AL, Freitas MPM. In vitro evaluation of microbial contamination of orthodontic brackets as received from the manufacturer using microbiological and molecular tests. Angle Orthod. 2015;85:992–6.
21. Rashid T, Wilson C, Ebringer A. The link between ankylosing spondylitis, Crohn's disease, Klebsiella, and starch consumption. Clin Dev Immunol. 2013;2013:1–9.
22. Rashid T, Ebringer A. Autoimmunity in rheumatic diseases is induced by microbial infections via crossreactivity or molecular mimicry. Autoimmune diseases. 2012;2012:1–9.
23. Sajjan P, Laxminarayan N, Kar PP, Sajjanar M. Chlorhexidine as an antimicrobial agent in dentistry–a review. Oral Health Dent Manag. 2016;15:93–100.

24. Hussein F, Hashem M, Chalisserry E, Anil S. (2014). The impact of chlorhexidine mouth rinse on the bond strength of polycarbonate orthodontic brackets. J Contemp Dent Pract. 2014;15:688–92.
25. Speer C, Zimny D, Hopfenmueller W, Holtgrave EA. (2005). Bond strength of disinfected metal and ceramic brackets: an in vitro study. Angle Orthod. 75:836–42.
26. Cheung H-Y, Wong MM-K, Cheung S-H, Liang LY, Lam Y-W, Chiu S-K. (2012). Differential actions of chlorhexidine on the cell wall of Bacillus subtilis and Escherichia coli. PLoS One. 7:e36659.
27. Borzabadi-Farahani A, Borzabadi E, Lynch E. (2014). Nanoparticles in orthodontics, a review of antimicrobial and anti-caries applications. Acta Odontol Scand. 72:413–7.
28. Hassan KA, Jackson SM, Penesyan A, Patching SG, Tetu SG, Eijkelkamp BA, et al. (2013). Transcriptomic and biochemical analyses identify a family of chlorhexidine efflux proteins. Proc Natl Acad Sci. 110:20254–9.
29. Donskey CJ, Deshpande A. (2016). Effect of chlorhexidine bathing in preventing infections and reducing skin burden and environmental contamination: a review of the literature. Am J Infect Control. 2016;44:e17–21.
30. Lambert P. (2002). Cellular impermeability and uptake of biocides and antibiotics in gram-positive bacteria and mycobacteria. J Appl Microbiol. 92:46S–54S.

CITATION OF THIS ARTICLE

Sunil K N, Manik S, Hrushikesh A, Vivek S, Mrunal A, Sejal S, Akshay C, Akshay B: Comparative Evaluation Of Microbial Contamination Of Orthodontic Microimplants And Assessment Of The Disinfecting Efficiency Of Chlorhexidine, Isopropyl Alcohol And Uv Light On Them - An Invitro Study. Bull. Env.Pharmacol. Life Sci., Vol 12[8] July 2023: 213-218.