ORIGINAL ARTICLE

The Effect of Repeated Immersion of Maxillary Acrylic Complete Denture Prosthesis in Sodium Hypochlorite Solution Before Trimming, in Controlling Microbial Load – a Randomized Controlled Trial.

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ABSTRACT

Objective

Trimming of complete denture poses a risk of cross infection to the dental professionals performing the adjustment. The conventional disinfection protocol involves immersion of the denture in a 0.5% sodium hypochlorite solution for 20 minutes before starting the procedure. The present study seeks to assess the effectiveness of a revised disinfection protocol, which involves immersing dentures in a 1% sodium hypochlorite solution for one minute repeatedly between adjustment procedures, alongside the conventional disinfection protocol, in order to control microbial load.

Materials and Methods

hirty-six maxillary complete denture patients were randomly divided into two groups, Group I conventional disinfection protocol and Group II repeated immersion disinfection protocol. Initially swab was taken from the prosthesis in both the groups to determine the microbial load and they were then subjected to conventional disinfection protocol. Following this, dentures in Group I were subjected to three cycles of trimming and swabs were again collected. In Group II, prostheses were subjected to intermittent one minute immersion in 1% sodium hypochlorite before each trimming cycle. After completion of trimming process swabs were taken. Blood agar and Mac Conkey agar were used to identify the organisms.

Results

Pretest – Posttest comparison by Wilcoxon signed rank test revealed statistically significant reduction of Klebsiella, Staphylococci and Streptococci in both Group I and Group II. Comparison of percentage reduction of microbial load in Group I and Group II was done using Mann-Whitney U test. The repeated immersion protocol resulted in a statistically significant reduction of Streptococci and Klebsiella when compared to conventional protocol.

Conclusion

Repeated one minute immersion in 1% sodium hypochlorite before each denture trimming cycle is effective in controlling the microbial load. The study was registered as a randomised clinical trial in the Clinical Trial Registry- India and listed as CTRI/2024/04/065264 in April 2024 Clinical Relevance: -Microbial recontamination of the denture occurs during adjustment procedures due to multiple reinsertions. Hence short repeated immersion disinfection protocols are advantageous in reducing microbial count and thereby alleviates the risk of cross infection.

Keywords

Disinfection, Sodium hypochlorite, Cross contamination, Immersion, Acrylic dental prosthesis

INTRODUCTION

The prosthodontic dental operatory is often prone to cross-contamination and potential disease transmission. For the safety of the patient and oral health professional, one of the main goals is to break the chain of infection transmission. Complete denture prosthesis is one of the most traditional methods of rehabilitation, for patients with complete edentulism. A removable prosthesis requires periodic adjustments immediately following insertion and in the later stages to fit in the ever-changing dynamic oral environment. Polymethyl methacrylate is the primary material ofchoice for denture bases with a track record of over 70 years, ease of use, low cost, and favorable physical and mechanical properties. However, an acrylic prosthesis in the

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oral environment absorbssaliva and oral fluids leading to color instability, increased water sorption, and porosity, resulting in contamination of the prosthesis [1]. Surface properties such as hardness, surface free energy, and surface chemical composition can influence bacterial adhesion onto the prosthesis [2]. Thesurface roughness of the dental materials ought to be maintained below 0.2 micrometers to decrease microbial attachment [3]. Another key factor that influences adhesion is wettability, which is determined by contact angle measurement. Wettability influences how the functional groups on the surface of the biomaterial, are rearranged when it comes in contact with a cell [4]. A water contact angle of 40° to 70° is optimal for cell attachment on polymers [5]. Additionally, bacteriaadhere more readily to hydrophilic surfaces than to hydrophobic ones, with the former exhibiting higher surface free energy values [6].

Bacterial adherence on acrylic prosthesis isan indicator of the porosity of the prosthesis [7]. A dental prosthesis in the mouth can act as a carrier for pathogenic organisms such as Gram-positive aerobes *(Streptococcus* spp. and coagulase- negative *Staphylococcus* spp.), Gram-positive anaerobes *(Actinomyces* spp. and *Klebsiella* spp.) as well as fungi (*Candida)* [8,9]. Disinfection procedures are mainly aimed at removing microbes from superficial layers. Mechanical trimming of the prosthesis exposes the deeper layers and the bacteria-laden acrylic splatter produced during trimming can contaminate the operator as well as the surroundings. The aerosols produced during polishing of these dentures may be inhaled or can come in contact with the skin, resulting in systemic and local infections.

Conventionally the dentures are disinfected prior to carrying out any adjustments to prevent cross -contamination to the clinicians and assistants. Various chemical disinfecting agents have been suggested in literature, such as sodium hypochlorite (NaOCl) at varying concentrations, 3.3-10% castor oil, 2% glutaraldehyde, 4% chlorhexidine gluconate etc. NaOCl andglutaraldehyde are the most commonly used denture disinfectants. Glutaraldehyde, when comes in contact with skin or eyes, can cause local irritation. Various concentrations of NaOCl and different immersion periods have been proposed, i.e., 0.25% and 0.5% for 20 minutes [10,11], 1%

for 10 to 15 minutes, 5.25% for 5 minutes [12,13]etc. with a maximum duration not exceeding 30 minutes.

NaOCl is a strong alkali with a pH above

11. This high pH disrupts the bacterial cell membrane, giving it antimicrobial property. It reacts with organic tissues by three main mechanisms - saponification, amino acid neutralization and chloramination [14]. Denture adjustment procedure requires multiple insertions and removal of the prosthesis from the mouth. Even though the prosthesis is subjected to conventional disinfection protocol of immersion in0.5% NaOCl solution for 20 minutes prior to adjustment, there is surface recontamination of the denture during reinsertions. This can pose a chance of cross infection to the dental professional performing the adjustment. Hence, a repeated immersion protocol, utilizing 1% NaOCl for one minute each, in between the adjustments seems tobe a viable option.

The current study aims to compare the effectiveness of two disinfection methods for controlling microbial load on maxillary acrylicdental prosthesis: the conventional protocol (20minutes immersion in 0.5% NaOCl) and the repeated immersion protocol (initial 0.5% NaOCl disinfection followed by one-minute immersion in 1% NaOCl before each of the three trimming cycles). It was carried out as a randomized controlled trial to minimize selection bias and to provide a clear analysis of how the intervention affects the results. Null hypothesis stated that there would be no difference in the effectiveness of conventional protocol versus repeated immersion protocol in controlling microbial load before trimming of maxillary acrylic dental prosthesis.

MATERIALS AND METHODS

The study was approved by Institutional Ethics Committee, Government Dental College, Thiruvananthapuram, Kerala, India with IEC Approval No. DCT/IEC/CT/24/20 dtd 15/03/2024. It was registered as a randomised clinical trial in the Clinical Trial Registry- India and listed as CTRI/2024/04/065264. Written consent was obtained from all participants before starting the clinical trial. All the study procedures followed theprinciples of Helsinki declaration on Human experimentation and abided by the Good Clinical Practice guidelines.

In the Department of Prosthodontics at Government Dental College, Thiruvananthapuram a randomised controlled trial was conducted using a double blind, during the period April 2024 to June 2024. Completely

edentulous patients wearing maxillary complete dentures for a continuous period of more than 2 months were selected for the trial. Individuals with a history of severe or chronic respiratory disease, infections of the oral cavity's soft tissues, or those who have taken systemic antibiotics within the last two months, sinonasal cavity, nasopharynx and larynx; uncontrolled chronic systemic diseases like diabetes, immunosuppressive states like retroviral infection; chemotherapy for malignancy; individuals who received organ transplant and are on immunosuppressants; individuals who have undergone irradiation for oromaxillofacialmalignancies and patients with xerostomia were excluded from the study. The research proceeded to choose patients in a sequential fashion until the sample size was reached, provided that they met all inclusion and exclusion criteria and gave their permission.

Sample size calculation

Sample size was calculated by assigning a power of 80%, an alpha error of 0.05, and an effect size of 1.5 [15]. There were a total of 32 participants, 16 from each group. In order to compensate for the loss of patients, a 10 % of the sample size was added to the original sample size making a total of 36 participants. A flow chart of the study participants is presented in Fig. 1.

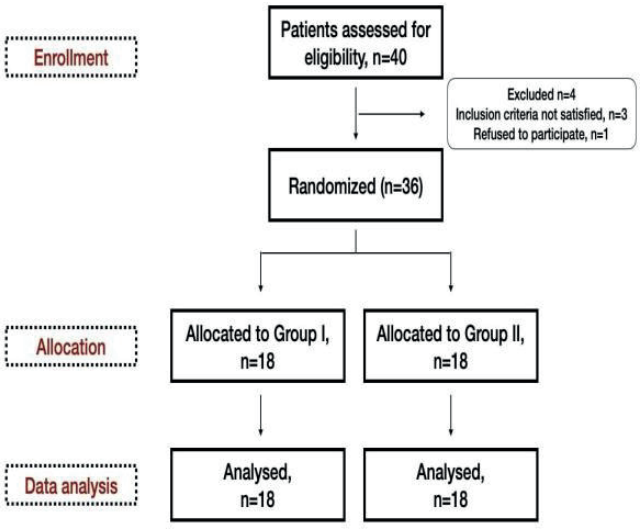

Fig. 1 Flowchart of study participant

Randomization and blinding

Randomization sequence was generated by the statistician using block randomization to balance the number of patients in each group. The treatment assigned was enclosed into sequentially numbered opaque envelopes and was kept with thedental hygienist, who was unaware of the study objectives. The NaOCl solution at the desired concentration was prepared by the dental hygienist according to the envelope selected. Neither the patient nor the outcome assessor was aware of thedisinfection protocol that was employed.

Thirty-six study participants were randomized into two groups, Group I and Group II, with each group comprising of 18 patients. Denture prosthesis was immersed in 200 ml of theNaOCl disinfectant solution. NaOCl 0.5%, was prepared by mixing 20 ml with 5% NaOCl with

180 ml of water. NaOCl 1% was obtained bycombining 40ml of 5% NaOCl with 160 ml of water.

1. Group I: Conventional disinfection protocol of the maxillary complete dentureprosthesis – One time immersion in 0.5% NaOCl solution for 20 minutes prior todenture adjustment procedures.

2. Group II: Repeated immersion disinfection protocol of the maxillary complete denture prosthesis - Conventional disinfection protocol followed by immersion in 1% NaOCl solution for one minute prior to each of the three trimming cycles.

To evaluate the initial microbial load, prior to disinfection, dentures in both Group I and GroupII were rubbed in a rolling fashion, with a sterile cotton-tipped swab moistened with phosphate buffered saline (PBS) along the intaglio surface, borders and polished surfaces of the dentures and then plated on Blood agar and Mac Conkey agar. Following this the dentures were subjected to conventional disinfection protocol. The prostheses were then washed in 200 ml distilled water to remove the NaOCl residue.

In Group I, the dentures were inserted in the mouth for one minute, followed by trimming for one minute using tungsten carbide bur on a micromotor (M3 Champion-Clinical Micromotor, Korea), at a speed of 35000 rpm. Dentures were washed in distilled water to remove acrylic debris. Two more cycles of insertion and trimming were done to mimic the adjustment procedures done in a clinical scenario. At the end of the third trimming cycle, swab was again collected from the dentures to assess the residual microbial load. The same evaluator conducted all the adjustment procedures to ensure uniformity. The co-investigator used a stop watch for monitoring the time.

In Group II after insertion in the mouth forone minute, the dentures were additionally subjected to immersion in 1 % NaOCl solution forone minute before trimming. This was repeated twice and at the end of third trimming cycle swab was taken to assess the residual microbial load.

Microbiological analysis was performed to isolate and identify the gram positive and gram- negative bacteria and *Candida* species. Quadrant streaking was done and agar plates were incubated for 24-48 hours and colony count was measured.

RESULTS

The data's normality was checked using the Shapiro-Wilk Test; a p value<0.05 indicated that the data did not follow a normal distribution. Therefore, the median and interquartile range (IQR) were used to represent continuous values. Group I and Group II colony counts werecompared before and after disinfection and trimming using the Wilcoxon Signed-rank test dueto the non-parametric nature of the data. Group II'spercentage decrease in microbial load was compared to Group I's using the Mann-Whitney Utest. Statistical significance was defined as a p- value lower than 0.05. We used SPSS 26.0, the Statistical Package for the Social Sciences, to conduct our statistical analysis.

BloodAgar	Group I		Wilcoxon signed- rank Test p	Group II		Wilcoxon signed-rank Test p
	Before disinfection Medi an (IQR)	After conventional method		Before disinfection	After Repeated immersion Protocol	
KLEBSIELLA (n=18)	50(12.5 - 200)	7(0 - 25)	0.001 *	50(13.25 - 92.5)	0(0 - 10)	0.001 *
E COLI (n=3)	200	2	0.18	112.5	0.5	0.180
PSEUDOMONAS (n=4)	15(0 - 157.5)	10(0 - 42.5)	0.18	15(2.5 - 35)	0(0 - 7.5)	0.109
ASPERGILLUS (n=1)	3	0	-	4	0	-
STAPHYLOCOCCI (n=15)	35(15 - 70)	8(5 - 20)	0.001 *	30(16 - 80)	4(2 - 20)	0.001 *
STREPTOCOCCI (n=18)	200(100 - 200)	200(77.5 - 200)	0.018 *	200(95 - 200)	50(37.5 -200)	0.002 *
CANDIDA	200	60		200	200	-

Staphylococci, Streptococci and Klebsiella were the most common organisms found on blood agar before disinfection in both group I and group

II. *E.Coli, Pseudomonas, Aspergillus and Candida* species were also identified in few cases. Mac Conkey agar was used to specifically identify the gram-negative organisms. There was nodiscernible growth of streptococci in Mac Conkey media. Wilcoxon signed-rank test showed a significant reduction in *Klebsiella, Staphylococci and Streptococci* in both Group I and Group II in blood agar when the colony counts before disinfection and after their respective disinfection protocols were compared (Table 1). Similar resultswere observed for *Klebsiella* in Mac Conkey agar(Table 2).

Table 1. Comparison of colony count of different microbial species (Blood agar) before and after use of conventional (Group I) and repeated immersion(Group II) disinfection protocols Statistically significant * p <0.05

The percentage reduction in microbial growth in Group I and Group II, analyzed throughMann-Whitney U test, provided insights into the comparative effectiveness of the two protocols(Table 3 and 4). In comparison to Group 1, Group II displayed a statistically significant

Table 2. Comparison of colony count of different microbial species (Mac Conkeyagar) before and after use of conventional (Group I) and repeated immersion (Group II) disinfection protocols

Mac ConkeyAgar	Group I		Wilcoxon signed Rank Test p	Group II		Wilcoxon signed Rank Test p
	Before disinfection Median (IQR)	After conventional method		Before disinfection	After Repeated immersion Protocol	
KLEBSIELLA (n=18)	44(10.25 - 125)	6.5(1.5 - 35)	0.001*	47.5 (12 - 80)	1(0 - 8.5)	0.001*
E COLI (n=3)	85	20	0.18	150	35.5	0.180
PSEUDOMONAS (n=4)	150(55 - 200)	32.5(19.75 - 70)	0.068	65(15.75 - 171.25)	6(0 - 12)	0.068
ASPERGILLUS (n=0)	0	0		0	0	
STAPHYLOCOCCI (n=15)	10(4 - 30)	2(0 - 6)	0.018*	12(3 - 30)	2(0 - 5)	0.050
STREPTOCOCCI (n=18)	0	0	0.317	0	0	
CANDIDA	0	0		0	0	

Statistically significant * p <0.05

percentage decrease in the microbial count of both Streptococci and Klebsiella in blood agar (Table 3). *Streptococci* decreased by 56.3 percentage in group II, while there was no reduction in group I, showing that the repeated immersion protocol is more effective than the conventional one.

Klebsiella, on the other hand demonstrated a median percentage reduction of 100% in Group II compared to 80% in Group I. Mac Conkey agar also evinced a statistically significant reduction for *Klebseilla* in Group II. *Staphylococci* did not display any significant percentage reduction inmicrobial count when both the protocols were compared.

Table 3: Percentage reduction in colony count in Group I and Group II in Blood agar.

Blood Agar	Percentage reduction		Mann-Whitney U testp
	Group I Median (IQR)	Group II Median (IQR)	
KLEBSIELLA (n=18)	80(68.8 - 96)	100(86.9 - 100)	0.008*
E COLI (n=3)	82	99.8	0.121
PSEUDOMONAS(n=2)	54.2	100	0.128
ASPERGILLUS(n=1)	100	100	1.00
STAPHYLOCOCCI (n=15)	60(45.5 - 83.3)	85(55.6 - 87.5)	0.056
STREPTOCOCCI(n=18)	0(0 - 35)	56.3(0 - 75)	0.029*
CANDIDA (n=1)	7P0erce	ntag0e	0.317
Mac Conkey	reduction		Mann-Whitney U test p
	Group I Median (IQR)	Group II Median (IQR)	
KLEBSIELLA (n=18)	77.5(51.8 - 94)	96.9(79.4 - 100)	0.007*
E COLI (n=2)	76.5	64.8	0.564
PSEUDOMONAS (n=4)	58.8(24.4 - 88.3)	97(78.5 - 100)	0.081
ASPERGILLUS(n=0)			
STAPHYLOCOCCI (n=12)	86.7(70.8 - 100)	85(43.1 - 92.5)	0.364
STREPTOCOCCI(n=0)			
CANDIDA (n=0)			

Statistically significant * p <0.05

Table 4: Percentage reduction in colony count in Group I and Group II in Mac Conkey agar Statistically significant * p <0.05

Box and Whisker plots showing percentage reduction in microbial count of *Streptococci (Fig.2), Klebsiella (Fig. 3) and Staphylococci (Fig. 4)* help visualize the differences between group I and group II, with the respective medians of each plot being compared. If the median line of one box plot falls outside the box of the other box plot, it likely indicates a difference between the two groups.Accordingly, *Streptococci and Klebsiella* showed a significant reduction in percentage microbial count in Group II when compared to Group I.

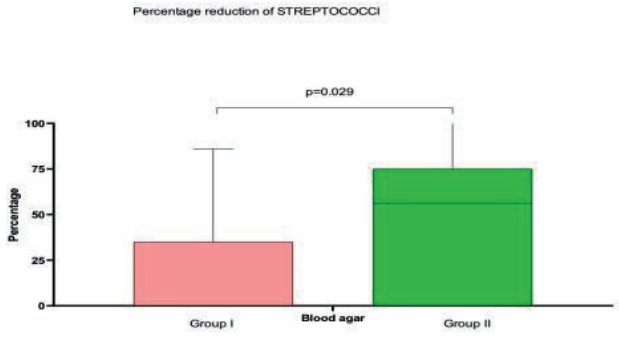

Fig.2 Percentage reduction of Streptococci in Group I and Group II in Blood agar

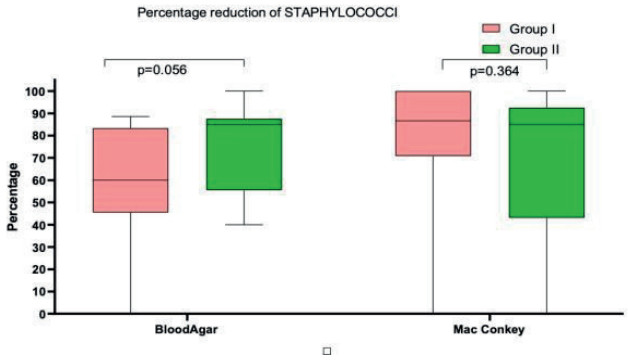

Fig.3 Percentage reduction of Klebsiella in Group I and Group II in Blood agar and Mac Conkey agar

Although not statistically significant, *E. coli and Pseudomonas* displayed higher median percentage reductions in Group II (99.8% and 100%, respectively) compared to Group I (82% and 54.2%, respectively) in blood agar. *Aspergillus and Candida* showed complete inhibition in both the groups.

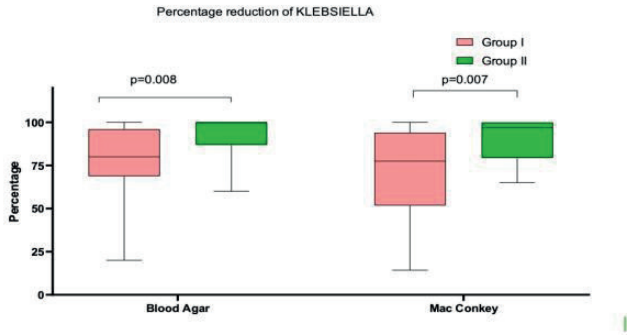

Fig.4 Percentage reduction of Staphylococci in Group I and Group II in Blood agar and Mac Conkey agar

DISCUSSION

Cross contamination in the prosthodontic dental operatory can result from direct contact withthe splatter or through inhalation of aerosols generated during trimming of denture. Even a contaminated lathe can result in cross infection during polishing of sterile dentures [16]. Aerosolsare tiny airborne particles smaller than 50 micrometres (µm), while splatter consists of larger particles over 50 µm in diameter [17]. Since the aerosol particles are smaller in size, they remain airborne for a prolonged period [16] and may be inhaled into the respiratory tract, thus serving as apotent source for transmission of infection. Sahanaet al. assessed the extent of aerosol spread in a prosthetic dental lab during trimming of acrylic dental prosthesis and found that the greatest concentration of aerosol particles was detected onblood agar plates kept 2 feet away from the lathe, indicating a high risk of transmission of infection to the dentist and lab technicians [18]. Allison et al. evaluated the aerosol and splatter following dental procedures and found that the greatest concentration was recorded at a distance of 1.5 meters, but aerosol particles can be dispersed up to4 meters [19]. Splatter on the other hand, stays airborne momentarily and settles down onto surfacescausing cross infection through direct contact

The presence of maxillary complete dentures in the oral cavity alters the oral environment and provides a favorable abode for microorganisms to grow. The various physiological activities like mastication and swallowing and the inherent cleansing ability of saliva easily detaches the organisms from the oralmucosal surfaces. But the mucosa underneath a denture is deprived of this natural washing processand the roughened contour of the intaglio surface of the denture can promote the

adhesion, aggregation and growth of microbial colonies which form a denture biofilm [20]. Attempting denture adjustments without previous disinfection can result in cross infection to the operator. Hence it is mandatory to adhere to strict denture disinfection during adjustment procedures. Various disinfection measures have been put forward including chemical disinfection, microwave and ultraviolet irradiation [21]. Immersion in a chemical disinfectant seems to be more predictable and reasonable compared to the other two modes.

In the present study, microbial colony count after conventional and repeated immersion protocols were compared. The repeated immersion protocol using 1% NaOCl for one minute was suggested under the assumption that recontamination of the prosthesis can result from repeated insertions during clinical adjustments. NaOCl at 1% concentration was used for disinfection of resilon cones with a one minute immersion time [22]. But complete denture prosthesis needs a longer immersion time for disinfection. However, since the dentures were initially disinfected for 20 minutes with 0.5% NaOCl and its impractical to extend disinfection time during the procedure, the study used one minute disinfection time. As immersion periods as long as 10 min has been recommended for 1% NaOCl, the repeated immersion protocol can be safely used without any adverse effect on the physico-mechanical properties of the acrylic denture base.

Pretest and Posttest comparisons within Group I and Group II showed a statistically significant reduction for *Streptococci, Staphylococci and Klebsiella*. Inter group comparison for percentage microbial load showed a statistically significant reduction in *Klebsiella* after repeated immersion protocol in both culture media. *Streptococci* also showed a statistically significant reduction after repeated immersion protocol in blood agar. *Staphylococci* did not demonstrate statistically significant reduction when both the protocols were compared. The findings of the study suggest that repeated immersion protocol is more effective than conventional protocol in reducing the microbial count, thus highlighting the potential benefits of this disinfection protocol.

As in a natural tooth, the denture once placed in the mouth gets coated with an 'acquired pellicle' comprising of salivary glycoprotiens and immunoglobulins [23]. This pellicle facilitates the adhesion of microorganisms. Composition of denture plaque can vary between fitting and exposed surfaces of the denture, but the predominant microbiota include S*treptococcus spp, Staphylococci spp, Gram- positive rods, Actinomyces spp, Lactobacilli, Propionibacterium, Veillonella, Gram-negative rods and yeasts* [16]. In the intaglio surface, S.*mutans and S. mitis* are more prevalent because of the acidogenic nature of the denture plaque in this area. It can be seen that the dentures examined in the study were thoroughly contaminated with *Streptococcus* species, *Staphylococcus* species and gram-negative facultative anaerobes like *Klebsiella*.

Streptococcus mutans are usually found on the hard, nonshredding surfaces of the mouth like teeth or dentures and have been isolated from cases of infective endocarditis. *Staphylococci* on the other hand are common microflora of skin in near vicinity to the mouth and the mucous layer of the nose. They are not commonly found in large numbers in the oral cavity but are invariably associated with denture plaque, in patients with denture stomatitis and in immunocompromised patients [24]. *Klebsiella and E. Coli* isolated from denture plaque are implicated in the development of bacterial pneumoniae [25]. Polishing of previously used denture can contaminate the polishing wheel and pumice resulting in carry over of oral flora to subsequent prosthesis. Kahn et al [26] have demonstrated that scrubbing with 3% hexachlorophene cleanser for one minute before polishing of adjusted denture resulted in substantial reduction of contamination. Although previous studies have proven the use of NaOCl at concentrations of 1% for an immersion period of 10min [27-29] for denture disinfection, the chances of recontamination following multiple insertions have not been addressed. But in this study repeated one minute immersion in 1% NaOCl [22,30] has been employed. NaOCl in concentrations of 0.5% to 1% has been found to be biocompatible and an immersion period upto 10 minutes does not have any deleterious effects on hardness and surface roughness of the acrylic prosthesis[31]. Most of the previous studies on the efficacy of NaOCl were in vitro experiments or clinical studies following one time immersion for the stipulated period of time. Randomized controlled trials have focused on long term or overnight immersion protocols [31-34], the results of which are not relevant in the context of the current study. This randomized trial thus signifies the importance of short, repeated immersions in

NaOCl disinfectant in controlling bacterial recontamination of dentures during adjustment procedures.

It should be noted that viruses can also be carried by the aerosols generated during shaping and trimmimg of dentures. In this study, the effectof repeated immersion protocol on reducing viral load was not evaluated. The research focussed on a limited group of complete denture wearers who visited the Prosthodontic clinic, and partially edentulous patients were not considered. Additionally, only one disinfectant was assessed; further studies should compare other disinfectant solutions and include a broader category of patients with diverse flora.

CONCLUSION

Repeated one minute immersion of acrylic dental prosthesis in 1% NaOCl before each denture trimming cycle has resulted in a statistically significant reduction in the colony counts of *Streptococcus* and *Klebsiella*. While *Staphylococci* displayed a reduction in colony counts, this change was not statistically significant. In contrast, *E. coli* and *Pseudomonas*, though present in fewer cases, demonstrated a greater percentage reduction in colony counts with repeated immersion. This signifies the importance of using the repeated immersion protocol in controlling recontamination of dentures during adjustment procedures. From a clinical perspective, the repeated immersion disinfection protocol offers a viable and cost-effective solution for controlling cross infection during trimming of dentures. The effect of repeated immersion protocol on viral load remains unexplored in this research

Declaration form

This work is my/our intellectual property, and I/we acknowledge that my/our name will be included as a contributor because I/we had a significant hand in coming up with the idea, designing it, analysingand interpreting the data (if relevant), and writing the manuscript. In my opinion, the manuscript is a legitimate piece of work. There is no publishing history or current plans for publication of this article or any other manuscript that has substantially comparable material and is authored by either of us. Not only is all of the data included in the publication, but no data from the research has been or will be published independently. I/we affirm this by signing here.

Author contribution

Dr. Kavitha Janardanan and Dr. Noxy George Manjuran contributed to the study conception and design. Dr. Prasanth V aided in material preparation, data acquisition and analysis. Statistical analysis and data interpretation was done by Dr. Harsha Kumar K. Dr. Vivek V Nair and Dr. Sreelakshmy K.S contributed to manuscript preparation, manuscript editing andreview. All authors read and approved the final manuscript.

Competing interests: The authors declare no competing interests.

Compliance with Ethical Standards: There are no potential conflicts of interest. The research was conducted in acrylic complete denture prostheses of human participants and informed consent was obtained from all the participants before the start of the study.

Ethics Approval: This research followed all applicable ethical guidelines, including those of the institutional review board andthe 1964 Helsinki Declaration and its subsequent revisions or equivalent ethical standards, and all procedures involving human subjects were carefully planned and executed. The research received the go light from the IEC, Govt Dental College, Thiruvananthapuram, with IEC Approval No. DCT/IEC/CT/24/20 dtd 15/03/2024

Conflict of interest: The authors declare that they have no conflict of interest.

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REFERENCES

1. Lin JJ, Cameron SM, Runyan DA, Craft DW (1999) Disinfection of denture base acrylic resin. J Prosthet Dent 81:202-6. doi: 10.1016/s0022-3913(99)70249-0. PMID:9922434.
2. Yoda I, Koseki H, Tomita M, Shida T, Horiuchi H, Sakoda H, Osaki M (2014) Effectof surface roughness of biomaterials on Staphylococcus epidermidis adhesion. BMC Microbiol 14:1–7. doi: 10.1186/s12866-014-0234-2.
3. Teughels W, Van Assche N, Sliepen I, Quirynen M (2006) Effect of material characteristics and/or surface topography on biofilm development. *Clin Oral Implant. Res.* 17:68–81. doi: 10.1111/j.1600-0501.2006.01353.x.
4. Fan H, Guo Z (2020) Bioinspired surfaces withwettability: Biomolecule adhesionbehaviors. *Biomater. Sci.* 8:1502–1535.doi: 10.1039/C9BM01729A.
5. Xu L, Siedlecki C.A (2007) Effects of surface wettability and contact time on protein adhesion to biomaterial surfaces. *Biomaterials.* 28:3273–3283. doi: 10.1016/j.biomaterials.2007.03.032.
6. Song F, Koo H, Ren D (2015) Effects of material properties on bacterial adhesion and biofilm formation. *J. Dent. Res.* 94:1027– 1034. doi: 10.1177/0022034515587690.
7. Lai CP, Tsai MH, Chen M et al (2004) Morphology and properties of denture acrylic resins cured by microwave energy and conventional water bath. Dent Mater 20:133 41.
8. Iosif L, Țâncu AMC, Amza OE, Dimitriu B, Ispas A, Pantea M, Imre M (2024) Qualitative assessment of the removable denture microbiome. Germs 2024 14:28-37. doi: 10.18683/germs.2024.1415. PMID: 39169976; PMCID: PMC11333846.
9. Pavarina AC, Pizzolitto AC, Machado AL, Vergani CE, Giampaolo ET (2003) An infection control protocol: effectiveness of immersion solutions to reduce the microbial growth on dental prostheses. J Oral Rehabil 30:532-6. doi: 10.1046/j.1365-2842.2003.01093. x. PMID: 12752936.
10. Salles MM, Oliveira Vde C, Souza RF, Silva CH, Paranhos Hde F (2015) Antimicrobial action of sodium hypochlorite and castor oil solutions for denture cleaning - in vitro evaluation. Braz Oral Res 29:1-6. doi: 10.1590/1807-3107BOR-2015.vol29.0104. Epub 2015 Aug 21. PMID: 26313346.
11. Badaró MM, Salles MM, Leite VMF, Arruda CNF, Oliveira VC, Nascimento CD, Souza RF, Paranhos HFO, Silva-Lovato CH (2017) Clinical trial for evaluation of Ricinus communis and sodium hypochlorite as denture cleanser. J Appl Oral Sci 25:324-334. doi:10.1590/1678-7757-2016-0222. PMID: 28678952; PMCID: PMC5482256.
12. Rudd RW, Senia ES, McCleskey FK, Adams ED Jr (1984) Sterilization of complete dentures with sodium hypochlorite. J ProsthetDent 51:318-21. doi: 10.1016/0022- 3913(84)90212-9. PMID: 6584598.
13. Manumeet Kaur Bhathal,Urvashi Kukreja,Navneet Kukreja (2018) Evaluation of Efficacy of Different Denture Disinfectants on Biofilms Formed on Acrylic Resin. Dental Journal of Advance Studies 6:20-27. Doi:10.1055/s-0038-1671696
14. Estrela C, Estrela CR, Barbin EL, Spanó JC, Marchesan MA, Pécora JD (2002) Mechanismof action of sodium hypochlorite. Braz Dent J13:113-7. doi: 10.1590/s0103-64402002000200007. PMID: 12238801.
15. Debattista, Neville & Zarb, Mario & Portelli, J.M (2010). Bacterial cross-contamination between the dental clinic and laboratory during prosthetic treatment. Malta Medical Journal. 22:12 - 14.
16. Agostinho AM, Miyoshi PR, Gnoatto N, Paranhos Hde F, Figueiredo LC, Salvador SL (2004) Cross-contamination in the dental laboratory through the polishing procedure of complete dentures. Braz Dent J 15:138-43.doi: 10.1590/s0103-64402004000200010.Epub 2005 Mar 11. PMID: 15776197.
17. Micik RE, Miller RL, Mazzarella MA, Ryge G (1969) Studies on dental aerobiology. I. Bacterial aerosols generated during dental procedures. J Dent Res 48:49-56. doi:10.1177/00220345690480012401. PMID:4887699.
18. Robert C. Kahn, Michael V. Lancaster, William Kate (1982) The microbiologic cross-contamination of dental prostheses. J ProsthetDent 47:556-559.
19. Allison JR, Currie CC, Edwards DC, Bowes C, Coulter J, Pickering K, Kozhevnikova E, Durham J, Nile CJ, Jakubovics N, Rostami N,Holliday R (2021) Evaluating aerosol and splatter following dental procedures: Addressing new challenges for oral health care and rehabilitation. J Oral Rehabil 48:61-72. doi: 10.1111/joor.13098. Epub 2020 Oct 8. PMID: 32966633; PMCID: PMC7537197.
20. Ahmed N, Abbasi MS, Haider S, Ahmed N, Habib SR, Altamash S, Zafar MS, Alam MK. Fit accuracy of removable partial denture frameworks fabricated with CAD/CAM, rapid prototyping, and conventional techniques: A systematic review. BioMed Res Int.2021;2021:3194433. doi:10.1155/2021/3194433.
21. Felton D, Cooper L, Duqum I, Minsley G, Guckes A, Haug S, Meredith P, Solie C, AveryD, Deal Chandler N (2011) American College of Prosthodontists. Evidence-based guidelines for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists. J Prosthodont 1:

S1-S12. doi: 10.1111/j.1532- 849X.2010.00683. x. PMID: 21324026.

22. Zand V, Salem-Milani A, Shahi S, Akhi MT, Vazifekhah S (2012) Efficacy of different concentrations of sodium hypochlorite and chlorhexidine in disinfection of contaminated Resilon cones. Med Oral Patol Oral Cir Bucal 17:352-5. doi: 10.4317/medoral.17467. PMID: 22143703; PMCID: PMC3448329.
23. Chawhuaveang DD, Yu OY, Yin IX, LamWY, Mei ML, Chu CH (2021) Acquired salivary pellicle and oral diseases: A literature review. J Dent Sci 16:523-529. doi: 10.1016/j.jds.2020.10.007. Epub 2020 Oct 24.PMID: 33384841; PMCID: PMC7770358.
24. Marsh P. D, Martin M. V, Lewis, M. A. O and Williams D. W (2009) Oral microbiology, 5th. Churchill Livingstone Elsevier, Edinburgh.
25. Sumi Y, Miura H, Sunakawa M, Michiwaki Y, Sakagami N (2002) Colonization of denture plaque by respiratory pathogens in dependent elderly. Gerodontology 19:25-9. doi: 10.1111/j.1741-2358.2002.00025. x. PMID: 12164235.
26. Sahana Kritivasan, Nazia Zareen. I, N.P. Muralidharan (2019) Assessing the extent of aerosol spread in prosthetic dental lab. INTERNATIONAL JOURNAL OF SCIENTIFIC & TECHNOLOGY Research 8:3190-3192.
27. Jayasoman S, D AP, K J. Effect of Silica Nanoparticles On Flexural Strength And Surface Hardness Of Heat Polymerized Acrylic Resin. Bangladesh J Med Sci. 2024;23(10):S79-S86
28. Pawashe KG, Tewary S, Sanyal PK, Nilesh K (2017) An In vitro Comparative Evaluation of Disinfectants on Standard and Clinical Microbial Strains on Heat Cure Resins. J Clin Diagn Res 11: ZC54-ZC58. doi: 10.7860/JCDR/2017/24759.9866. Epub 2017 May 1. PMID: 28658908; PMCID: PMC5483810.
29. Orsi IA, Junior AG, Villabona CA, Fernandes FH, Ito IY (2011) Evaluation of the efficacy of chemical disinfectants for disinfection of heat-polymerised acrylic resin. Gerodontology 28:253-7. doi: 10.1111/j.1741-2358.2010.00400. x. Epub 2010 Jul 1. PMID:20609007.
30. Dumani A, Yoldas O, Isci AS, Köksal F, Kayar B, Polat E (2007) Disinfection of artificially contaminated Resilon cones with chlorhexidine and sodium hypochlorite at different time exposures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 103: e82-5. doi: 10.1016/j.tripleo.2006.10.013. Epub 2007 Jan 22. PMID: 17241794.
31. Peracini A, Regis RR, Souza RF, Pagnano VO, Silva CH, Paranhos HF (2016) Alkaline Peroxides Versus Sodium Hypochlorite for Removing Denture Biofilm: a Crossover Randomized Trial. Braz Dent J 27:700-704. doi: 10.1590/0103-6440201600913. PMID: 27982182.
32. Koshy E, Annamma LM, Idrissi HA, Abutayyem H. Management of a Failed Hybrid Implant Full-arch Prosthesis-A Case Report. Bangladesh J Med Sci.2024;23(1):266-71.
33. Arruda CNF, Salles MM, Badaró MM, de Cássia Oliveira V, Macedo AP, Silva-Lovato CH, de Freitas Oliveira Paranhos H (2017) Effect of sodium hypochlorite and Ricinus communis solutions on control of denture biofilm: A randomized crossover clinical trial. J Prosthet Dent 117:729-734. doi:10.1016/j.prosdent.2016.08.035. Epub 2016Dec 4. PMID: 27927282.
34. Newaskar PS, Sonkesriya S, Singh R, Palekar U, Bagde H, Dhopte A. Evaluation and Comparison of Five-Year Survival of Tooth-Supported Porcelain Fused to Metal and All- Ceramic Multiple Unit Fixed Prostheses: A Systematic Review. Cureus. 2022 Oct 15;14(10):e30338.