

A Comparative Analysis of the Effect of two Mouthrinses on the Accumulation of Biofilm on Dentures.

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Abstract

Introduction: One of the common problems among denture wearers is the formation of plaque on the surfaces of the dentures. For many patients mechanical denture hygiene measure may be insufficient to achieve plaque removal on the dentures hence the rationale for the use of mouth rinses. Thus, this study aimed at comparing the effect of two mouth rinses on the accumulation of biofilm on dentures.

Methodology: Patients were selected through a simple random sampling method (balloting) and subsequently assigned into 3 study groups; chlorhexidine digluconate group (CHX), hexetidine group and control group (normal tap water) with each group consisting of 43 patients. All the patients attended thrice: day 1 (baseline), day 7 (1st recall) and day 14 (2nd recall). Stained surface analysis was done using Image processing software 2012 (Image J tool 3.0 for Microsoft windows). Further descriptive and inferential analysis was done using IBM SPSS version 20.0 and test of statistical significance was done using Analysis of variance (ANOVA). The confidence level was set at 95% and the P-value of 0.05 or less was taken as statistically significant.

Result: Mean percentage biofilm coverage area for CHX group was 31.43%, hexetidine group was 29.58% while that of control group was 54.55%. The ANOVA result showed that the mean difference between percentage biofilm coverage area of CHX and hexetidine groups was not statistically significant (P=0.215), whilst the difference between the experimental groups (CHX and hexetidine) and the control group was statistically significant (P=0.001)

Conclusion: The tested denture cleansing agents used in this study, CHX and hexetidine solutions were equally efficacious in reducing biofilm and were superior to the control agent (water).

Keywords: Partial dentures, biofilm, CHX digluconate, hexetidine, water, oral hygiene, denture hygiene.

I. Introduction

The insertion of a removable prosthesis in the mouth results in significant changes in the oral environment which may lead to adverse effects on the integrity of the oral tissues.¹The oral health status of denture wearers is often poor and several studies have observed the **deteriorating status** of the oral health of denture wearers.^{2,3} Poor oral hygiene in denture wearers can be associated with lack of guidance, intrinsic characteristics of dentures and diminished manual dexterity of denture **users**, especially in old age.⁴ It is absolutely essential to ensure that the patient is trained or instructed about the importance of maintenance of denture hygiene and that the patient is recalled at regular intervals to ensure that the oral hygiene is maintained.^{5,6} Poor denture hygiene results in the accumulation of biofilms which makes adequate **cleaning** mandatory for denture wearers.^{5,6} Biofilms are dense microbial layer formed by microorganisms and their metabolites.⁷ They usually consist of more than 10 microorganisms per gram of dry weight.⁷ **Biofilms** can be formed by a single bacterial specie, but more often consists of many species of bacteria, as well as fungi, algae and protozoa.⁷

Biofilms form on dental prostheses and appliances such as mouth guards and night guards,⁸ and these appliances can become colonized with large numbers of microorganisms within hours. Biofilm accumulation can lead to a host of local and systemic problems.⁹ These include bad breath, acrylic resin pigmentation, stains, formation of calculus deposits as well as development of chronic atrophic candidiasis or denture stomatitis.⁵ Organism associated with denture biofilms can sometimes spread and cause infections of the lungs and gastrointestinal organs.¹⁰ Biofilms can also be responsible for diseases which can be highly resistant to antibiotics.⁷ This can increase the risk of developing dental caries and periodontal diseases especially on the abutment teeth that retain the dental prosthesis in the mouth.⁸ Accumulation of plaque on dentures is a major problem for denture wearers (partial or complete; upper or lower; immediate or **definitive**). It is generally

accepted that poor maintenance of hygiene in most denture wearers occurs not only in compromised geriatric patients, but also in younger and healthy patients. **This may** be attributed to a lack of motivation due to poor knowledge of the clinical importance of denture hygiene or just carelessness and neglect.¹¹

Mechanical and chemical methods are available for denture cleaning. Mechanical methods can be classified into brushing (with water, soap, detergents and abrasives) and ultrasonic devices. These methods are however mostly inadequate due to reduced manual dexterity in elderly patients and poor access to grooves and crevices in the denture with a mechanical cleaning **device.** The chemical methods make use of substances such as denture mouth rinses (like CHX, hexetidine, triclosan and mineral agents), hypochlorites, peroxides, neutral peroxides with enzymes and acids.¹¹ There is a great popular acceptance for mouth rinses mainly due to their pleasant fragrance and fresh taste.¹²

Observations and studies have also shown that poor oral hygiene and poor denture hygiene are common in this country and that chemical agents are not widely used in denture care.¹³ The purpose of this study was thus to evaluate the effectiveness of a simple cleansing protocol for denture wearers, which advocates the use of commonly recommended and commercially available mouth rinses which are 0.2% CHX digluconate (Corsodyl) and 0.1% hexetidine (Hexedene).

II. Methodology

The Lagos State University Teaching Hospital (LASUTH) is a referral Centre for majority of Lagos residents hence it is open to the general public. Lagos State has an estimated population of about 17.5 million,¹⁴ with a population growth of about 3.2% per **annum.** Every month an average of about 1,300 (One thousand three hundred) patients attend the Dental clinic.

This study was a randomized controlled double-blinded clinical trial comparing the effect of two mouth rinses. A comparison was made on the effect of the two mouth rinses on the accumulation of biofilm on dentures worn by partially dentate patients referred to the Prosthetics unit of the Restorative Dentistry Department of LASUTH. These included males and females between the ages of 20 and 70 years.

Permission to carry out the research was obtained from the Health Research and Ethics Committee (HREC) of the Lagos State University Teaching Hospital (LASUTH). The consent of each patient was obtained before being included in the study and the patients who agreed to participate in the study were assured of their confidentiality. There was no undesirable consequence for not participating in the study.

An interview-administered questionnaire was used to obtain background information from the subjects. The information obtained included the age, gender, duration of denture usage and the cause of tooth loss. Others include where the denture was made, history of any recent lesion in the mouth, use of mouth rinse during the period of the lesion, history of use of mouth rinse, type of mouth rinse, length of time, history of systemic condition (diabetes and hypertension), presence of pregnancy (females), smoking pattern and history of allergy to any drug.

A total of one hundred and twenty nine patients were selected through simple random sampling and subsequently assigned into 3 study groups; chlorhexidine digluconate group, hexetidine group and control group (normal tap water) with each group consisting of 43 patients. The patients were instructed to soak their dentures for 10 minutes in the provided solution for each group and then rinse and immerse in water overnight before wearing it in the morning throughout the study period. All the patients attended thrice: day 1 (baseline), day 7 (1st recall) and day 14 (2nd recall). At each visit, the dentures were rinsed with water and stained with disclosing agent (hydrated dextrates and magnesium stearate). The dentures were soaked in the disclosing solution for ten minutes. The stains on the fitting surface (buccal, lingual or palatal) of the denture were photographed using a digital camera. The images were analyzed using Image processing software 2012 (Image J tool 3.0 for Microsoft windows). After the biofilm had been quantified, the percentages were calculated and the data analyzed using IBM SPSS version 20.0 and test of statistical significance was done using Analysis of variance (ANOVA). The confidence level was set at 95% and the P-value of 0.05 or less was taken as statistically significant.

III. Results

Each study subject received their denture at the base line during their first visit to the clinic. On the second visit (1st recall), 43 subjects each from the hexetidine and control groups were reviewed while 42 respondents from the chlorhexidine group were seen (one subject was lost to follow-up). At the third visit (2nd recall), 41 subjects in the CHX group were reviewed, compared to all 43 in the hexetidine group and 42 in the control group were seen.

Table 1 shows that majority of the respondents were in the age group of 46 – 71 years. All the groups also had a similar distribution of males and females. Majority of the subjects also had a tertiary level of education (65.1%) and most of them were self-employed (38.8%). There was no statistically significant difference between demographic characteristics of the subjects in the three groups ($P > 0.05$).

Majority of the subjects in the three groups were first time denture users (42.6%) and most of them had no previous mouth lesion (90.7%) (Table 2) while trauma was the commonest aetiology of tooth loss among the study subject (59%) - Figure 1

There was no history of hypertension or diabetes mellitus among the study subjects and only 3.1% of them were current smokers (Table 3).

Table 4 shows that the mean overall biofilm percentage coverage area for chlorhexidine group was 31.43% (± 4.86); 29.58% (± 3.17) for the hexetidine group while that of control group was 54.55% (± 5.06) as shown under image J analysis tool. Multivariate analysis (ANOVA) showed that the difference in mean percentage coverage area between the two experimental groups and the control group was statistically significant ($P = 0.001$).

The mean difference between CHX and hexetidine group was not statistically significant. ($P = 0.215$) However, the mean difference between chlorhexidine and control group ($P = 0.002$) as the mean difference between hexetidine and control group was statistically significant ($P = 0.000$).-Table 5

IV. Discussion

Denture plaque biofilm is a sheltered community of microbial growth that allows survival in a hostile environment.¹⁵ It is a structured community of microorganisms with a high cell density enclosed in a self-produced polymeric matrix that is adherent to an inert or living surface.¹⁶ Dentures create an ideal environment for the adhesion and growth of pathogenic organisms which are harmful to the periodontium and surrounding teeth. Inadequate cleaning of dentures can make them to act as a reservoir of microorganisms which continuously recolonize the oral cavity even after oral hygiene has been performed. Cleanliness of the dentures is of paramount importance to prevent oral diseases among denture wearers.^{17,18} For effective cleansing of the denture, it is critical to remove plaque (biofilm) not only from the polished surfaces of the prosthesis but more importantly, the tissue fitting surface.¹⁹ There are several physical and chemical techniques for removing biofilms from dentures and the use of chemical solutions is one of the effective methods of improving denture hygiene.

In this study more than one third of the respondents (42.6%) were first time denture users and most of the participants (59%) reported that trauma due to accidents and assaults were the major cause of their loss of teeth. This was in contrast to previous studies carried out among some Nigerians and other Africans that observed that periodontal disease was a major cause of tooth loss.²⁰⁻²⁵ The urban nature of the study location and a rise in prevalence of orofacial injuries due to trauma and assault may be responsible for this trend. None of the respondents had any previous history of systemic conditions and majority of the respondents (96.9%) were non-smokers and (3.1%). Overall, 51.6% of all the subjects were females while 48.4% were males. This may be due to the fact that females pay more attention to their oral care than the males.

Findings from this present study showed that the overall biofilm mean percentage coverage area for CHX group was 31.43%; hexetidine group was 29.58%, while that of control group (H₂O) was 54.55%. The difference in mean percentage coverage area between the experimental groups and the control group was statistically significant. The mean difference between CHX and hexetidine group was however not statistically significant ($P = 0.215$). This showed that both CHX and hexetidine had relatively close effectiveness on biofilm reduction and that both of them had a greater inhibitory effect than water. A similar study which corroborated the efficacy of these two mouth rinses was that by Himratul et al,²⁶ which demonstrated that supragingival micro flora could be successfully suppressed by the use of oral rinses which was judged by the reduction in colony forming units (cfu) of the supragingival micro flora. It was clearly shown that both agents were effective in reducing dental plaque microbes.

Chlorhexidine gluconate has a broad-spectrum antimicrobial activity and it is a relatively safe oral antiseptic with antifungal and antibacterial action. Its substantivity in the oral cavity which prolongs its therapeutic effect, enables it to bind to salivary pellicles as well as hard tissues in the oral cavity, resulting in chlorhexidine titres in saliva for 12 hours or more after rinsing.²⁷ The oral preparation is in the form of chlorhexidine gluconate, which is a water-soluble compound, with a physiological pH that is dissociable, allowing the release of positively charged ions²⁸ which attaches to the negative bacterial charge. Chlorhexidine's bactericidal effect occurs through cell membrane lysis and cytoplasmic precipitation.²⁹ Pavarina et al³⁰ observed that 4.0% chlorhexidine was very effective in reducing denture biofilm accumulation after 10 minutes of immersion when they compared its effectiveness to that of 1.0% sodium hypochlorite, iodophors, and alkaline peroxide. Topical use of chlorhexidine may however have some deleterious effects, especially with prolonged use. These include superficial staining of the teeth and other oral structures, an increase in calculus formation and alteration in taste perception.³¹

Hexitidine **is also** a bactericidal and fungicidal cationic antiseptic with a wide spectrum of actions against gram positive and gram negative bacteria, as well as some fungi and parasites. It is used as a 0.1% mouthwash for local infections and oral hygiene. Some authors have observed a variable effect of hexetidine on

biofilm formation in the oral cavity³² while some other researchers observed a very positive effect.³³ Brown coloration of the denture resins soaked in other antiseptics like chlorhexidine has not been described with hexetidine. Some brands of Hexetidine are also cheaper than chlorhexidine and this could be cost effective for indigent patients.

Even though none of the mouth rinses completely cleared the biofilm to 0% level and the percentage of coverage of biofilm was slightly high for both CHX and hexetidine, the percentage of biofilm formation was still significantly lower than in the control group.

V. Conclusion

This present study showed that the tested denture cleansing solutions namely CHX digluconate and hexetidine were equally efficacious in reducing accumulation of denture biofilms and were both superior to the control method which was the use of water. The afore-mentioned mouth rinse solutions are potentially valuable complements to mechanical plaque control and hence can contribute to maintenance of the oral health care of denture wearers.

Limitation

Culture of organisms and anti-microbial analysis not done in this study. This could have provided further evidence on the effectiveness of Hexetidine and chlorhexidine. This could however serve as a basis for further studies.

Table 1: Socio-demographic characteristics of the study subjects.

Variables	GROUPS			Total n=43 (%)
	Chlorhexidine Groupn=43 (%)	Hexetidine Groupn=43 (%)	Control Groupn=43 (%)	
Age (yrs)				
20 – 45	16 (37.2)	17 (39.5)	13 (30.2)	46 (35.7)
46 – 71	27 (62.8)	26 (60.5)	30 (69.8)	83 (64.3)
Mean ±SD	52.00±5.86yrs	50.37 ±7.23yrs	54.86 ±7.64yrs	52.43 ±6.92yrs
Sex				
Male	23 (53.5)	22 (51.2)	18 (41.9)	63 (48.4)
Female	20 (46.5)	21 (48.8)	25 (58.1)	66 (51.6)
Educational Level				
No Formal Edu.	2 (4.7)	0 (0.0)	1 (2.3)	3 (2.3)
Primary	1 (2.3)	2 (4.7)	2 (4.7)	5 (3.9)
Secondary	1 (27.9)	15 (34.9)	10 (23.2)	37 (28.7)
Tertiary	28 (65.1)	26 (60.4)	30 (69.8)	84 (65.1)
Occupation				
Civil servants	3 (7.0%)	8 (18.6%)	3 (7.0%)	14 (10.9)
Professionals	13 (30.2%)	14 (32.6%)	13 (30.2%)	40 (31.0%)
Self-employed	18 (41.9%)	14 (32.6%)	18 (41.9%)	50 (38.8%)
Unemployed	2 (4.6%)	4 (9.2%)	6 (13.9%)	12 (9.2%)
Others	7 (16.3%)	3 (7.0%)	3 (7.0%)	13 (10.1%)

TABLE 2: Dental history of study subjects.

Variables	GROUPS			TotalFreq. (%)
	Chlorhexidine GroupFreq. (%)	Hexetidine GroupFreq. (%)	Control GroupFreq. (%)	
Denture of usage duration				
First User	18 (41.9)	21 (48.8)	16 (37.2)	55 (42.6)
< 5 years	13 (30.2)	12 (27.9)	10 (23.3)	35 (27.1)
≥ 5 years	12 (27.9)	10 (23.3)	17 (39.5)	39 (30.3)
Total	43 (100.0)	43 (100.0)	43 (100.0)	43 (100.0)
Previous Mouth Lesion				
Yes	4 (9.3%)	3 (7.0%)	5 (11.6)	12 (9.3)
No	39 (90.7%)	40 (93.0%)	38 (88.4%)	117 (90.7)
Total	43 (100.0)	43 (100.0)	43 (100.0)	43 (100.0)
Time of last Lesion/s (weeks)				
< 4 weeks ago	1 (25.0)	2 (66.7)	2 (40.0)	5 (41.7)
> 4 weeks ago	3 (75.0)	1 (33.3)	3 (60.0)	7 (58.3)
Total	4 (100.0)	3 (100.0)	5 (100.0)	12 (100.0)
Use of Mouth rinse during the period of the observed Lesion/s				
Yes	0 (0.0%)	1 (33.3%)	2 (40.0%)	3 (25.0%)
No	4 (100.0%)	2 (66.7%)	3 (60.0%)	9 (75.0%)
Total	4 (100.0)	3 (100.0)	5 (100.0)	12 (100.0)

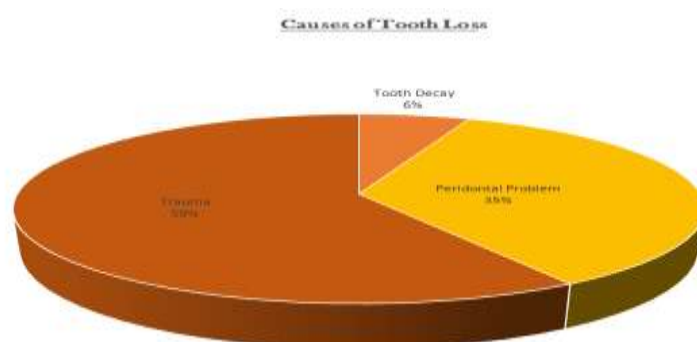


Figure 1: Chart showing causes of tooth loss among subjects.

Table 3: History of systemic conditions and lifestyle of the subjects.

Variables	GROUPS			TotalFreq. (%)
	Chlorhexidine GroupFreq. (%)	Hexetidine GroupFreq. (%)	Control GroupFreq. (%)	
Previous history of Diabetic				
Yes	0 (0.0)	0 (0.0)	(0.0)	0 (0.0)
No	43 (100.0)	43 (100.0)	43 (100.0)	129 (100.0)
Total	43 (100.0)	43 (100.0)	43 (100.0)	129 (100.0)
Previous history of Hypertension				
Yes	0 (0.0)	0 (0.0)	(0.0)	0 (0.0)
No	43 (100.0)	43 (100.0)	43 (100.0)	129 (100.0)
Total	43 (100.0)	43 (100.0)	43 (100.0)	129 (100.0)
Smoking habits				
Smokers	2 (4.7)	1 (2.3)	1 (2.3)	4 (3.1)
Non-smokers	41 (95.3)	42 (97.7)	42 (97.7)	125 (96.9)
Total	43 (100.0)	43 (100.0)	43 (100.0)	129 (100.0)
Number of sticks smoked per day.				
None	41 (95.3)	42 (97.7)	42 (97.7)	125 (96.9)
< 10 sticks	2 (4.7)	1 (2.3)	1 (2.3)	4 (3.1)
> 10 sticks	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	43 (100.0)	43 (100.0)	43 (100.0)	129 (100.0)
History of drug allergy				
Drug Allergy	15 (34.9)	11 (25.6)	14 (32.6)	40 (31.0)
Non Drug Allergy	28 (65.1)	32 (74.4)	29 (67.4)	89 (69.0)
Total	43 (100.0)	43 (100.0)	43 (100.0)	129 (100.0)

Table 4: Mean percentage of biofilm coverage on removable dentures for each group.

Mean Coverage Area per Visit	Chlorhexidine (1st Group)	Hexetidine (2nd Group)	Control (3rd Group)	P-value
Baseline	00.00±0.00	00.00±0.00	00.00±0.00	0.001*
First Recall	31.44% ±4.13	30.73% ±2.91	54.25% ±5.01	
Second Recall	31.43% ±3.58	28.43% ±3.30	54.88% ±5.42	
Total Average	31.43% ±4.86	29.58% ±3.17	54.55% ±5.06	

Table 5: Multiple comparisons of the mean percentage of biofilm coverage on removable dentures for Test and Control groups.

Group Comparism	Mean Difference between groups	Standard Error	P-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Groups 1 and 2	1.847121	1.485919	.215	-1.07934	4.77358
Groups 1 and 3	-23.129370	1.490206	.002*	-26.06427	-20.19447
Groups 2 and 3	-24.976490	1.476997	.000*	-27.88538	-22.06760

*. The mean difference is significant at the 0.05 level.

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