Original Research Paper

Volume - 12 | Issue - 01 | January - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Prosthodontics

COMPARISON OF ANTIMICROBIAL PROPERTY OF DENTURE BASE RESIN INCORPORATED WITH CHLORHEXIDINE AND SILVER NANOPARTICLES- AN IN VITRO STUDY

Dr Sapna Rani* MDS, Reader, Dept of Prosthodontics, ITS-CDSR, Muradnagar, India. *Corresponding Author

Dr. Sangeeta Madaan MDS, Postgraduate Student, Dept of Prosthodontics, ITS-CDSR, Muradnagar, India.

ABSTRACT

Aim- To comparatively evaluate antimicrobial properties of silver nanoparticles and chlorhexidine incorporated in heat cure denture base resin.

Settings and design- Antimicrobial property was evaluated for each sample by measuring zone of inhibition around specimens and compared with another group.

Materials and Methods- 30 samples were fabricated for the study and sampling was done according to following criteria: Group 1- Control Group (No incorporation of any material in heat cure denture base resin), Group 2- Chlorhexidine incorporated in denture base resin (2% w/w) and group 3- Silver nanoparticles incorporated in denture base resin (2% w/w). Samples were inovulated in agar plates specific to Candida Albicans and incubated at 37˚C for 24 hours. Antimicrobial property was evaluated by measuring zone of inhibition around discs.

Statistical analysis- Descriptive statistical analysis was carried out. Mean and standard deviation was calculated and kruskal-willis non-parametric test was used to compare mean.

Results- The mean difference for group 1 (Control group) was 0.0+/- 0.0, for group 2 (Chlorhexidine in denture base resin) was 7.4+/- 0.56, for group 3 (Silver nanoparticles in denture base resin) was 0.0+/- 0.0.

Conclusions- There was significant difference in antimicrobial property of group 2 (Chlorhexidine in denture base resin) as compared to other groups.

KEYWORDS : Antimicrobial property, Chlorhexidine, silver nanoparticles, denture base resin

INTRODUCTION

It is well-known that removable denture bases fabricated from heat-polymerised acrylic resins may act as a reservoir for microorganisms and contribute to re-infection in denture wearers.[1] Biofilm deposition on the surface of acrylic denture bases is enhanced by the characteristics of the material, especially its porosity, irregularity and absorption.[2] Oral candidiasis is the most common infection involving oral mucosal tissues in complete denture wearers.[3] It is estimated that it affects about 72% of this population. Inspite of its multifactorial etiology, denture biofilm components, such as Candida albicans yeast, play a fundamental role in the development of Candidiasis.[4] The treatment of candidiasis includes denture repair or replacement, adoption of prophylactic measures by the patient and the prescription of antifungal drugs.

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. In the present scenario, nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties.[5] The metallic nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains.[6]

The silver nanoparticles show efficient antimicrobial property compared to other salts due to extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity.[7,8]

Chlorhexidine may be considered as an appropriate alternative to conventional antimycotic drugs in the management of oral candidiasis.[9] It has been reported that the duration of inhibition of adherence of candida was longer-lasting when chlorhexidine was used rather than amphotericin B and nystatin.[10] The antifungal effect of chlorhexidine has been shown in many studies, and it has been demonstrated that exposure of C. albicans tochlorhexidine suppresses the ability of candida to adhere to buccal epithelial cells. Immersion of acrylic dentures in chlorhexidine suppresses adhesion of candida to the prosthesis.[11]There are several studies showing antimicrobial property of individual material but there is no available data comparing the two materials. The purpose of this study was to compare the antimicrobial property of silver nanoparticles and chlorhexidine when incorporated in denture base. The Null hypothesis was that there was no difference in silver nanoparticles and chlorhexidine powder particles in terms of antimicrobial property.

MATERIALS AND METHOD

Three groups of samples of PMMA in the form of disc of diameter 3mm were prepared for the study: (1) Group 1: Control group (No incorporation of any material) (2) Group 2: PMMA samples with chlorhexidine powder (2%) (3) Group 3: PMMA samples with silver nanoparticles (2%).

Sample preparation

For group 1, samples were prepared by mixing polymer and monomer without incorporation of any other material in 3:1 ratio by volume. The mixing ratio and conditions for processing and polymerization recommended by the manufacturer were followed strictly.

For group 2, samples were prepared by incorporation of chlorhexidine 2% w/w within the PMMA dentu

For group 3, samples were prepared by incorporation of silver nanoparticles in a concentration of 2% w/w in powder measured by a weighing machine.

The wax patterns were fabricated with the help of putty index (Affinis, Coltene Whaledent) for standardization of all specimens. Investment of wax patterns was done in die stone. The tested materials were added in the specified ratio to the acrylic resin powder by a weighing machine (Unique instruments, Mumbai) (Fig 3). The mixture of powder and liquid monomer was then stirred for 15 seconds and left standing for 4 minutes until a plastic dough was formed. The dough was then packed into disc-shaped mould to produce a disc specimen (3 mm diameter and 2.0 mm thickness). After packing, the mould was allowed to stand for 30 minutes, and then placed in a curing unit and cured.[13] Samples were retrieved and finished.

Antimicrobial activity evaluation

Before antimicrobial activity evaluation, the specimens were autoclaved to rule out any microorganism. Samples were inoculated in high chrome agar plates and incubated at 37˚C for 24 hours for the growth of C. Albicans in incubator. Since the media is specific to C. Albicans, an inhibition zone will be created around the specimen who is active against C. albicans. The antimicrobial property was demonstrated by the occurrence of a growth inhibition zone around the wells containing the control, chlorhexidine-impregnated discs, silver impregnated discs, which was expressed by measurement of the diameter of the inhibition zone present around the well accommodating the drug-release device, using a divider and scale (Fig 1). The measurements were taken from centre of disc to outer diameter of inhibition zone. All readings were calculated and subjected to statistical analysis.

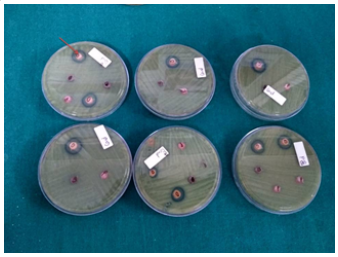

Fig 1- Zone of inhibition for Group 1, 2 and 3

STATISTICALANALYSIS

All readings were subjected to statistical analysis. Non parametric Kruskal-Willis test was applied to compare the mean. All calculations were performed using the SPSS (version 16) for windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Mean and standard deviation was calculated for each group and data was compared with Kruskal-Willis test. The mean difference for group 1 (Control group) was 0.0+/- 0.0, for group 2 (Denture base resin with chlorhexidine) was 7.4+/- 0.56, for group 3 (Denture base resin with silver nanoparticles) was 0.0+/- 0.0.

On comparison, it was found that there was significant difference in antimicrobial property of group 2 (Denture base resin with chlorhexidine) as compared to other groups (Table I).

Table I-NON – PARAMETRIC TEST (Kruskal – Wallis & Median Test)

Kruskal-Wallis Test

Ranks			
	Groups	N	Mean Rank
Outcome	Group 1	10	10.50
	Group 2 (Chlorhexidine)	10	25.50
	Group 3 (Silver nanoparticles)	10	10.50

Test Statistics[a,b]	
	Outcome
Chi-Square	27.663
df	2
Asymp. Sig.	.000
a. Kruskal Wallis Test	
b. Grouping Variable: Groups	

DISCUSSION

In this study, antimicrobial property of Chlorhexidine and Silver nanoparticles incorporated in denture base resin were evaluated and compared with negative control group. Due to reasons like limited motor skills and loss of memory of geriatric patients, incorporation of these particles was done in denture base resin rather than immersion denture in antifungal solutions. The release of chlorhexidine from the PMMA drug-release device into distilled water indicates that polymerization of the PMMA acrylic resin did not adversely affect the antifungal drug nor did impregnation of the PMMA acrylic resin with chlorhexidine alter the diffusion characteristics of the resin. So this is one of the methods for incorporation of Chlorhexidine in acrylic resin which is in agreement with that of previous studies using polymers for delivering chlorhexidine.[13]

It is well known that Ag ions and Ag-based compounds have strong antimicrobial effects.[14] Inorganic nanoparticles have a distinct advantage over conventional chemical antimicrobial agents. The most important problem caused by the chemical antimicrobial agents is multidrug resistance. Generally, the antimicrobial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism.[15] Since reducing the particle size of materials is an efficient and reliable tool for improving their biocompatibility and also reducing the size increases antimicrobial property, so silver nanoparticles were used in this study.

In the present in vitro study, Zone of inhibition was found only around chlorhexidine incorporated denture base resin which indicated strong antimicrobial property of chlorhexidine. It is an established fact that chlorhexidine solution is an effective medium for antimicrobial activity when denture is immersed in this solution.[16] Results of this study suggested that incorporation of chlorhexidine powder in polymer of acrylic resin is an effective medium for sustained release of drug. The results are in accordance with previous study by **D J Lamb et al.**[17]

There was no zone of inhibition around silver nanoparticles which contradicts previous studies by **L.A. Casemiro et al**[18]. The reason may be that they used zeolites instead of silver nanoparticles directly in polymer. Zeolites are aluminum silicate crystalline structures that present void spaces measuring 3–10 angstroms in their structure. Antimicrobial cations, such as silver and zinc, may be lodged within the void spaces of the zeolites and be exchanged over time with other cations from their environment. **K. Y. Nam et al**[15] also evaluated the antifungal activity of silver nanoparticles in denture base resin but antifungal activity was found at higher concentration.

The limitation with silver nanoparticle incorporated in denture base was discoloration of denture base resin even when used in low concentration. So other methods of incorporation of silver nanoparticles may be opted which maintains the esthetics of denture. There is scope of further studies in which concentration of silver nanoparticles can be increased with maintenance of esthetics.

CONCLUSION

Within the limitations of present in vitro study, the modified denture base acrylic combined with chlorhexidine at 2.0 wt% displayed antimicrobial properties. Chlorhexidine can be incorporated in routine dentures for its antimicrobial property.

Figure legends

Fig 1- Samples preparation of group 1, group 2 and group 3
Fig 2-2A Chlorhexidine base, 2B- Silver nanoparticles
Fig 3- Electonic weighing balance
Fig 4- Incubator
Fig 5- Zone of inhibition around discs

Table legends

Table 1- Mean and standard deviation of different samples
Table 2- Kruskal- willis test

REFERENCES

1. Radford DR, Challacombe SJ, Walter JD. Denture plaque and adherence of Candida albicans to denture-base materials in vivo and in vitro. Crit Rev Oral Biol Med1999; 10:99–116.
2. Sesma N,Lagana DC,Morimoto S,Gil C. Effect of denture surface glazing on denture plaque formation. Braz Dent J2005; 16:129–134.
3. Schneid TR. An in vitro analysis of a sustained release system for the treatment of denture stomatitis. Spec Care Dentist1992; 12:245–250.
4. Budtz-Jorgensen E.Oral Candidiasis in long term hospital care denture wearers with denture stomatitis. Oral Dis1996; 2:286–290.
5. Morones JR, Elechiguerra JL, Camacho A, Ramirez JT. The bactericidal effect of silver nanoparticles. Nanotechnology 2005; 16:2346–53.
6. Gong P, Li H, He X, Wang K, Hu J, Tan W, et al. Preparation and antibacterial activity of Fe3O4@Ag nanoparticles. Nanotechnology 2007; 18:604–11.
7. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions onEscherichia coliandStaphylococcus aureus. J Biomed Mater 2000; 52(4):662–8.
8. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. colias a model for gram-negative bacteria. J Colloid Interface 2007; 275:177–82.
9. Tobgi RS, Samaranayake LP, McFarlane TW. Adhesion of Candida albicans to buccal epithelial cells exposed to chlorhexidine gluconate. J Med Vet Mycol. 1987; 25(5):335–338.
10. McCourtie J, McFarlane TW, Samaranayake LP. A comparison of the effects of chlorhexidine gluconate, amphotericin B and nystatin on the adherence of Candida species to denture acrylic. J Antimicrob Chemother. 1986; 17(5):575–583.
11. Giuliana G, Pizzo G, Milici ME, Musotto GC, Giangreco R. In vitro antifungal properties of mouthrinses containing antimicrobial agents. J Periodontol. 1997; 68(8):729–733.
12. Ryalat S, Darwish R, Amin W. New form of administering chlorhexidine for treatment of denture-induced stomatitis. Therapeutics and clinical Risk Management 2017: 7;

219:225.
13. Riggs PD, Braden M, Patel M. Chlorhexidine release from room temperature polymerizing methacrylate systems. Biomaterials. 2000; 21(4): 345–351.
14. Furno F, Morley KS, Wong B, Sharp BL, Arnold PL, Howdle SM, et al. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection. J Antimicrob Chemother 2004; 54:1019 - 24.
15. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH. Antimicrobial property of silver nanoparticles. Nanomedicine 2007: 3(1); 95-101.
16. Sai pavithra .R, Muralidharan N.P. Antimicrobial Activity of Chlorhexidine and Iodine against Candida Species on Denture Base. J Pharm Sci Res 2015 ; 7(8): 600-601.
17. D.J Lamb and M. C. Martin. An in vitro and in viva study of the effect of incorporation of chlorhexidine into autopolymerizing acrylic resin plates upon the growth of Candida albicans. Biomaterials 1983; 3: 205-209.
18. Casemiro L A, Martins G H C, Panzeri C F, Pires-de-Souza, Panzeri H. Antimicrobial and mechanical properties of acrylic resins with incorporated silver–zinc zeolite – part I. Gerodontology 2008;25:187-194.