ORIGINAL ARTICLE

Antifungal Effect of Silver Nano Particles Coating on Denture Base Specimens Made of Acrylic Resin

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OBJECTIVE: The present study was under taken to determine the anti-fungal effect of Silver Nano partial coating in concentrations of 0.1%, 0.2%, 0.5% and 1% on heat cure acrylic denture through diffusion disc method. Poly methyl methacrylate (PMMA) or simply acrylic is most commonly used material for construction of complete dentures. Denture stomatitis is an inflammatory disorder of oral mucosa, frequently observed in denture wearers. The unpolished intaglio surface of PMMA base dentures coupled with adverse conditions such as poor hygiene, dry mouth and compromised immune system leads to denture related stomatitis in 50-70% of complete denture wearers. Antifungal agents such as silver have been added to acrylic denture bases to in part self-disinfect property.

METHODOLOGY: The supplied modeling wax sheet was cut in to 25 specimens of dimensions 10x10x 2mm with help of wax knife. The wax sheet was invested in stone plaster with in metal flask using open flasking method for mould formation. Heat cured acrylic resin polymer and monomer (Meadway Royale Heat Cure, MR. Dental, and UK) was mixed according to manufacturer's instructions of 2.5gm powder to 1ml monomer. The mixed acrylic was packed in dough stage followed by pressure packing in hydraulic bench press for 30 minutes under 9.8 MPa. Curing was done by placing the flask in water at room temperature until boiling. It remained in boiling water for 45 minutes and then allowed to cool down in water bath. A total of 25 acrylic plates were recovered from the flask and divided into five groups. Group A have no coating , group B coated with 0.1% sliver nano particles, group C coated with 0.2% silver nano particles, group D coated with 0.5% silver nano particles and group E coated with 1% silver nano particles solution. Each specimen was cut in to 6mm disc by Laser Engraving Machine. These discs were utilized for calculating zone of inhibition through diffusion disc method in agar media.

RESULTS: The diameter of zone of inhibition increased with the increasing concentrations of silver nano particles. When the concentration of silver nano particles was 1%, the zone of inhibition size was maximum (20.48mm). When the concentration was 0.1%, the size of zone of inhibition was minimum (10.02mm) .This difference was statistically found to be highly significant (0.005).

CONCLUSION: This study results demonstrate that silver nano-particles have good antifungal activity against Candida Albicans when used as surface coating. This antifungal property is directly influenced by the concentration of silver nano particles used.

KEY WORDS: Antifungal property, Silver nano particles (AgNPs), Candida Albicans (CA).

HOW TO CITE: Haider B, Imran M, Raza M, Riaz Z, Hanif A, Akram S. Antifungal effect of silver nano particles coating on denture base specimens made of acrylic resin. J Pak Dent Assoc 2022;31(2):59-64.

DOI: https://doi.org/10.25301/JPDA.312.59

Received: 17 June 2021, Accepted: 07 January 2022

INTRODUCTION

Edentulism is defined as irreversible condition resulting in loss of teeth affecting masticatory function, nutrition, speech, and esthetics.[1] Edentulisum is considered to be an effectual indicator for proficiency of a nation dedication toward their oral health care. Complete dentures weather tissue supported or implant supported are considered to be the most obvious rehabilitation technique for geriatric patients.[2]

Various treatment options for edentulism have been proposed. One of them is implant retained over denture. This denture is retained by dental implant. Disadvantages of implant retained over denture is that as it is supported by mandible, resorption of supporting structure will results in increase tipping of denture results in dislodgment. Another option is fixed hybrid prosthesis. Such dentures are fixed so

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patients cannot remove it for cleaning and repairing require dentist to removal and resetting.[3]

Acrylic removable complete dentures are considered to be treatment of choice for edentulisim due to good aesthetics, ease of manipulation and cost effectiveness.[4] Like any other material acrylic have inherent draw backs such as water sorption and release of monomer leading to low impact strength. Additionally the unpolished intaglio surface of heat cure acrylic resin dentures attract fungal growth in deep crevices, contributed by poor oral hygiene, dry mouth and compromised immune system intern leading to denture stomatitis.[5] According to Fonda et al denture stomatitis is 50-70% prevalent in complete denture wearers.[6] Mostly (78%) the denture stomatitis is associated with candida growth out of which 68% are Candida Albicans.[7] Prevention of denture related stomatitis includes orals rinses, mechanicals or chemical disinfection, denture replacement and educating the patient not to wear the denture overnight. Management of denture stomatitis also includes systemic or topical antifungal therapies.[8] Systemic anti-fungal therapies have disadvantage of recurrence and can induce side effects. Topical therapies are associated with microbial resistance development and their miss use or improper application leads to reduced efficiency.[9] Many of antifungal medicaments were used in tissue conditioner for treating denture stomatitis but these medicaments have antifungal effects for short period of time.[10]

In view of recent trend towards making material bioactive, various releasing and non-releasing anti-fungal agents have been added to acrylic denture base material.[11] Silver ions (Ag) have great antimicrobial proficiency with low toxicity and good biocompatibility with human tissues. Studies show long-term antimicrobial activity due to sustained ion release and low microbial resistance.[12] Since alloy powder act as bioactive fillers in resin matrix, they can be added to a limited concentration for it can affect the mechanical properties during use.[11]

Silver nano particles have been used as Coating of various instruments and implant prosthesis to avoid or at least decrease the microbial colonization without compromising the instruments and prosthesis mechanical properties.[13] Adhesion of biofilm formation is reduced in denture base resin due to silver nano particles coating.[1] In view of above review of literature, the present study proposes to use silver coating over intaglio surface of acrylic complete denture to impart anti-fungal properties in acrylic denture base material. The unpolished surface of acrylic plates was coated with different concentrations of silver ions to test the null hypothesis. It was considered that the silver coating will not induce antifungal activity at concentrations of 0.1%, 0.2%, 0.5% and 1%. Additionally time dependent anti-fungal behavior of these silver coated acrylic plates were assessed through Agar plate diffusion test method.

METHODOLOGY

A laboratory based experimental study was carried out to manufacture 25 heat cure acrylic plates. The wax patterns was fabricated with modeling wax (Metrowax Metrodent, Ltd. UK) having dimension of 2x 80x 40mm. A total 25 specimens of dimensions 10x10x2 mm were engraved into wax with help of wax knife. The mould was made by mixing 60gms of dental stone powder (manufacturer.) with 100gm of water. The mixed dental stone slurry was vibrated on vibrator to remove an entrapped air bubbles. Wax pattern was carefully placed on dental stone slurry surface and slightly pressed. Once the stone was set separating media was applied to facilitate the clean opening of open mould. After the application of separating media the wax pattern was completely invested in dental stone.[15]

Heat cure acrylic resin polymer and monomer (Meadway Royale Heat Cure, MR. Dental, and UK) was mixed according to manufacturer's instructions with 2.5gm powder to 1ml monomer in a ceramic container and covered with a lid to prevent evaporation of monomer. The mixture at dough stage was kneaded, rolled and packed into prepared gypsum moulds. Two trials closure were done by closing flask each time in hydraulic press at 9.8MPa. The heat cure acrylic flash was removed with scalpel blade no 15. The flask was finally closed and placed under 9.8MPa pressure in hydraulic press for 5 minutes before clamping Bench curing was done for 30 minutes after which the flask was placed in a water curing bath (Model:HH-S4SN:301171726) at room temperature and temperature was set to raise 1°C per minute until temperature reached 100°C that temperature was maintained for 45 minutes to complete curing cycle followed by bench cooling for half an hour and then immersed in water for 15 min prior to opening.[5]

The acrylic plate was recovered from the flask and was separate into 25 specimens of 10x10x2 mm. The excess of resin was removed gently by tungsten carbide using micro motor (Marathon SDE-M35LS Taiwan).Silicon Carbide sand paper (400-600-800 grit) was used for finishing. The finished specimens were polished using wet rag wheel and pumice slurry and then with Laser Engraving Machine (BCL-1610X) these specimens were cut in to round discs of 6mm.

The prepared specimens were checked for any imperfections, cracks, bends, visible porosities and fractures. The selected specimens were immersed in 5ml of distilled water in their respective sterile air tight containers for a period of 48 ± 2 hours to eliminate and release residual monomers if any.

Specimens were divided into five groups i.e. A, B, C, D and E having five specimens each. Group A specimens was not coated and assigned as control. Specimens in group B, C, D and E were coated with preformed AgNPs (Sigma Aldrich, Germany obtained from a local supplier) in the Single Wafer Spin Processor (WS-400E-6NPP-LITE) in physics department of Peshawar University in concentration of 0 .1%, 0.2%, 0.5% and 1% respectively.

Saboraud dextrose broth (SDB) was prepared by mixing 3.5mg SDB powder in 250ml distilled water (manufacturer's recommended ratio). The suspension was stirred until complete mix before it is autoclaved at 115°C under 1.5 bars pressure. The media was stored at 0°C as a stock in refrigerator till use (MIDAS international LB-LWB1.
5-5X33R).

The Candida Albicans standard strains were obtained from the laboratories of Pakistan Council Scientific and Industrial Research (PCSIR). The antifungal activity was evaluated by agar disc diffusion assay by measuring the zone of inhibition against test microorganism by using method of Kirby-Bauer.[16] A 48 hours culture of Candida Albicans was standardized to 0.5 McFarland's standard in a test tube using normal saline. The standard 0.5 McFarland's 10^6 CFU/ml of 100ul of standardized microbial culture was spread with help of glass spreader.

Saboraud dextrose agar (SDA) was prepared by mixing 8.2mg SDA powder in 125ml distilled water (manufacturer's recommended ratio). The suspension was stirred until complete mix before it is autoclaved at 115°C under 1.5 bars pressure. Laminar flow was cleaned with 70% ethanol solution and decontaminated with ultra violet light for 20 minutes. Sterile Petri plates were arranged in laminar flow and SDA was poured in Petri plates (10ml/plate). The prepared Petri-plates after sufficient hardening was stored at 0^oC as a stock in refrigerator till use

Various concentrations of AgNPs coating over discs by spin coater (MODEL WS-400BZ-6NPP/LITE). Group A specimens were not coated while Group B specimens coated with 0.1%, Group C specimens coated with 0.2%, Group D specimens coated with 0.5% and Group E specimens coated with 1%.

The antifungal activity was compared by zone of inhibition in different concentration of silver nano particles coating specimen.

Petri dishes that were prepared and having fungal growth were loaded with discs. Discs were placed on the surface of the agar, using sterile forceps. Once discs had contacted the agar surface they were not moved. Petri dishes were covered with lid to minimize exposure of the agar surface to room air and placed them in a 35°C air incubator for 1 hour, 3hours, 8hours and 12 hours.

DATA ANALYSIS PROCEDURE

The data was entered in to SPSS version 20 for analysis. Arithmetic means and SDs were calculated for zone inhibition of all specimens in Group A, Group B, Group C, Group D and Group E. For comparison of means of various groups for zone of inhibition, one-way analysis of variance (ANOVA) was used. P-value less than 0.05 was considered significant.

RESULTS

Specimens in group A were negative control with no coating of AgNPs. No zone of inhibition was observed when the specimens were checked after 1 hour, 3hours, 8hours and 12hours. For group B, heat cured PMMA acrylic resins coated with 0.1% AgNPs, the average Zone of inhibition was 10.02mm at 1 hr. This zone of inhibition was constantly maintained at 3hours, 8hours and 12hours (Figure 1). For specimens in group C, coated with 0.2% silver nano particles, the average zone of inhibition was 13.04mm after 1 hour, 3 hours, 8 hours and 12 hours. Heat cured acrylic resins used for fabrication of specimens in group D were coated with 0.5% silver nano particles. The average zone of inhibition was 15.04mm after 1 hour, 3 hours, 8 hours and 12 hours. The zone of inhibition for group D was maximal (20.04mm) which also remained constant after 1 hour, 3 hours, 8 hours and 12hours (Table 1).

Table 1: Average value and standard deviations

Groups	Mean zone of inhibition in millimeters (mm)					SD
	0 hr (baseline)	1 hr	3 hrs	8 hrs	12 hrs	
Group A (Control)	0	0	0	0	0	0
Group B	0	10.00	10.0	10.02	10.2	± 0.2
Group C	0	12.5	13	13	13.5	± 0.5
Group D	0	15.0	15.04	15.04	15.3	± 0.3
Group E	0	19.	20.48	20.6	20.48	± 0.6

When comparing the means of all groups (One-way ANOVA) revealed that the antifungal activities of specimens in experimental group B, C, D and E showed statistically significant results($p= 0.05$) as compared to negative control (group A) at all-time intervals (Table 2).

For multiple comparisons the specimens were paired in ten pairs as A-B, A-C, A-D, A- E, B-C, B-D, B-E, C-D, C-E, and D-E (Table 3). The analysis of multiple comparison revealed that the experimental groups B, C, D, and E showed a statistically significant difference in antifungal activity against Candida Albicans via agar diffusion test. Amongst all the groups the experimental Group E had elevated values of antifungal activity.

Table 2: One-way analysis of variance showing significance in between groups and various time intervals.

		ANOVA
		Sig.
0hr	Between Groups	0.003
	Within Groups	
	Total	
1 hr	Between Groups	0.001
	Within Groups	
	Total	
3 hrs	Between Groups	<0.001
	Within Groups	
	Total	
8 hrs	Between Groups	0.005
	Within Groups	
	Total	
12 hrs	Between Groups	0.004
	Within Groups	
	Total1	

Table 3: Multiple comparison between groups

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P-value
Group A	Group B	-10.0200*	.3127	0.004
	Group C	-13.0000*	.3127	0.005
	Group D	-15.0400*	.3127	0.001
	Group E	-20.4800*	.3127	0.003
Group B	Group A	10.0200*	.3127	0.002
	Group C	-2.9800*	.3127	0.001
	Group D	-5.0200*	.3127	0.004
	Group E	-10.4600*	.3127	0.003
Group C	Group A	13.0000*	.3127	0.005
	Group B	2.9800*	.3127	0.002
	Group D	-2.0400*	.3127	0.001
	Group E	-7.4800*	.3127	0.005
Group D	Group A	15.0400*	.3127	0.003
	Group B	5.0200*	.3127	0.004
	Group C	2.0400*	.3127	0.000
	Group E	-5.4400*	.3127	0.001
Group E	Group A	20.4800*	.3127	0.003
	Group B	10.4600*	.3127	0.005
	Group C	7.4800*	.3127	0.000
	Group D	5.4400*	.3127	0.001

*. The mean difference is significant at the 0.05 level

DISCUSSION

In the present study, it was observed that silver coating over acrylic did impart anti-fungal properties in acrylic denture base material. The unpolished surface of acrylic plates when coated with different concentrations of silver ions produced zone of inhibition that proportionately increased with increased concentration of silver in the coating. Thus the null hypothesis was rejected. Additionally time dependent anti-fungal behavior of these silver coated acrylic plates were assessed through Agar plate diffusion test method. This showed significant improvement at 1 hr subsequently at 3, 8 and 12 hr the anti-fungal effect was effectively maintained but no significant improvement was observed. Thus the maximal antifungal effect was expressed at 1 hour and found to be constant till 12 hour time.

Silver has been tested to be a potent anti-fungal agent. Correa et al[12] synthesized AgNPs by chemical reduction method. The incorporation of silver nano particles in tissue conditioner with concentration of 0.1%, 0.5%, 1%, 2% and 5% showed effective antimicrobial property.

Silver nano particles when incorporate in to polymethylmethacrylate PMMA show unpredictable outcome in regards to its physical properties. Some studies reports degradation of physical properties[17], while other studies show improvements in properties like surface roughness.[18]

The result of a study done by Chladek et al are in agreement with present work. As silver nano particles in both studies increase the antifungal effect.[19] To overcome the unpredictable outcomes of silver incorporation, Kagami et al used Argon (Ar) for implanting silver on PMMA. The PMMA plates were simultaneously implanted with both Fluoride (F) and Ag ions using or Ar/F gases and Ag mesh by a plasma -based ion implantation PBII process increasing surface contact angle by both ion implantations inhibiting the growth of S. mutants but no significant antifungal activity against C. albicans. The author in their study failed to provide any substantial reasons for poor anti-fungal property of AgNPs when used with argon deposition.[20]

Work done by Kamikawa et al. is in support of present study. Their work showed a high antifungal activity with no mechanical impairment of PMMA when coated with AgNPs. The thickness of specimens used in Kamikawa et al. work were not recommended for the dental denture bases. The present study has specimen made out in geometric shape, in accordance to the actual denture base thickness. Additionally in the present study the sustain anti-fungal behaviors or effect was also tested.[21]

Queiroz et al. treated surface of denture base material by chemical vapour deposition method, and reported decreased Candida Albicans bio film formation. However their work lack on time dependent performance of silver when coated through chemical vapour method.[22]

Khan et al. worked with silver nano particles synthesized from Aspergillus niger. Their results support the present work as the antifungal properties of AgNPs coating were profound.

In fact the zone of inhibition was almost 100mm.[23] The large zone of inhibition might be due to difference in process of synthesis of AgNPs. In their study the natural source for silver was used and the particles were freshly made and may be more active. In the present study commercially available particles were used. The present study particle size and surface activation both can be different from the Khan et al work.[24]

In one of study the different nystatin was added in to tissue conditioner having effective antifungal property but hardness and roughness increased with time. As roughness increased there is greater chance for growth of fungi leads to denture related stomatitis. This method decreases its antifungal property with time .The present study shows sustained antifungal effect.[25]

It's beyond the scope of this study to comment on such aspects as no such investigations were carried out. Never the less they also did not profile the effect of time on anti-fungal effect.

LIMITATIONS OF THE STUDY

The present work is an experimental study in which the surface characterization of samples coated with silver nano particles was not done. Scanning electron microscopy (SEM) is used for taking image before and after coating with silver nano particles for measuring particle distribution and silver nano particles were not treated for better adhesion.

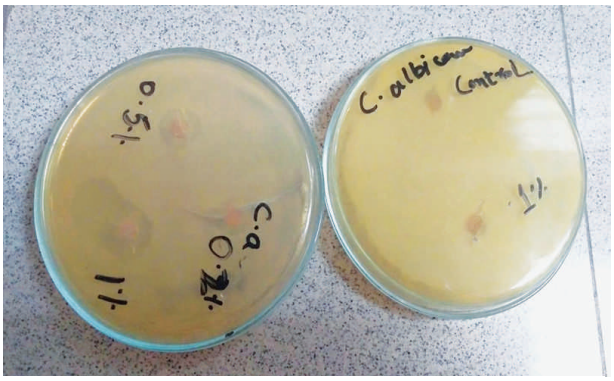
CONCLUSIONS

This study results demonstrate that silver nano-particles have good antifungal activity against Candida Albicans when used as surface coating. This antifungal property is directly influenced by the concentration of silver nano-particles used. Furthermore AgNPs coating on acrylic reached its maximal potential within 1 hr and could maintain it effectively for 12 hrs.

RECOMMENDATION

Since AgNPs have excellent anti-fungal effect, there is potential in further studying the characterization of cytotoxicity of such potent agent. Sensitivity of AgNPs coating to species other than Candida Albicans should be tested. Comparison of antifungal effect of other antifungal agents with antifungal effect of silver nano particles and silver nano particles activation potential according to source (natural or synthetic) should also be studied. Additionally the prolong anti-fungal effectiveness and integrity of this surface coating to mechanical challenges like wear should be studied.

Fig 1: Zone of inhibition



CONFLICT OF INTEREST

None declared

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