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Effect of silver nanoparticles incorporation on microhardness of Heat-cure denture base resins

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ABSTRACT

Background: Poly (Methyl methacrylic acid) based materials are widely used for the fabrication of removable complete and partial denture prosthesis. Regular cleansing of these dentures may abrade the surface due to an inherent lack of adequate surface hardness. This roughness may adhere food to the denture surface, making it dirty and further cause stomatitis. Numerous studies reported the antimicrobial activity of denture base materials incorporated with silver nanoparticles, which may logically prevent microbial growth on the denture. However, the effect of these nanoparticles on the mechanical properties, which provide longevity to the prosthesis, was not substantiated.

Aim: This study was designed to evaluate the effect of incorporating various concentrations of silver nanoparticles into heat-cure denture base resin materials, on their surface hardness. .

Materials and methods: A Silver nanoparticles were incorporated at various concentrations (0.5, 1.0, 2.0 and 5.0 wt%) into three different heat-cure denture base materials. A total of 150 rectangular-shaped specimens (62 x 10 x 2.5), which comprises 50 samples from each of the three heat-cure acrylic resins were made using the compression moulding technique. Ten specimens (n=10) were allocated for each concentration such as control, 0.5wt%, 1.0wt%, 2.0wt% and 5.0wt% concentrations of silver nanoparticles. The microhardness was evaluated using the Vickers microhardness tester. The data were subjected to One way ANOVA and Tukey HSD tests for statistical analyses.

Results: Significant differences (p=0.000) were observed in the surface hardness between the unmodified and modified denture base materials. .

Conclusion: Silver nanoparticles can be considered as the favourable additives to increase the surface hardness of denture base materials.

1. Introduction

Dentures remain the most popular choice of prosthetic devices with a high success rate for the treatment of edentulous conditions. Complete dentures are usually made with polymers, precious metal alloys and base metal alloys [1,2].

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Among these, polymer-based materials, especially Poly (methyl methacrylate) resins are widely used for the fabrication of complete and partial dentures [3,4]. The reasons for using PMMA resin as the most common materials are due to its favourable working characteristics, accurate fit, and stability in the oral environment, superior aesthetics, and ease of processing with inexpensive equipment. However, it has few shortcomings such as the frequent fracture of dentures due to mechanical fatigue and chemical degradation of the base material, low thermal conductivity [1-4] and ease of microbial adherence to the intaglio surface [5,6]. Numerous researchers have studied the effect of incorporation of various fillers like metallic particles, fibres, nanoparticles, etc., on the mechanical properties of PMMA materials with varying success [4]. Further, attempts have been made to copolymerize PMMA with rubber materials to improve the impact strength [4]. Though these attempts were successful to a certain extent, the problem of microbial adhesion remained as a critical feature of PMMA based denture base materials leading to Denture stomatitis.

Various studies reported the application of antifungal agents orally in the form of drops, lozenges, cream, pastille, lacquer, gel or mouthwashes [5-7]. However, these attempts reported the loss of the drug rapidly into saliva, inhomogeneous distribution of the drug, and the development of resistance to the antifungal therapy [6].

Recently, numerous studies have uncovered the effect of nanoparticle incorporation on the antimicrobial activity of the polymers. Among the various nanoparticles, silver nanoparticles (AgNPs) received a particular interest by the researchers as they show a broad spectrum of antimicrobial activity. Alla RK *et al.* [8] studied the antimicrobial activities against different microorganisms, and flexural strength [9] of heat-cure denture base materials incorporated with various concentrations of silver nanoparticles. Studies reported that the denture base materials incorporated with AgNPs exhibited antimicrobial activity against C.*Albicans*, and S. *Mutans*, especially at lower concentrations [8].

However, the effect of incorporation of AgNPs on the mechanical properties of denture base materials has not been validated. The surface hardness along with the strength is an essential mechanical property as denture prosthesis should exhibit maximum resistance to abrasion under masticatory forces and while cleaning dentures. Due to lack of adequate wear resistance, the surface of the dentures becomes rough during regular denture cleaning procedures. The food and debris may stick to these rough surfaces resulting in an unhygienic denture and thereby cause stomatitis [10]. Therefore, to prevent this adversity, it is necessary to increase the hardness of acrylic resins. Hence, this study aimed to evaluate and compare the surface hardness of heat-cure denture base resins modified with the incorporation of different concentrations of AgNPs.

2. Materials and methods

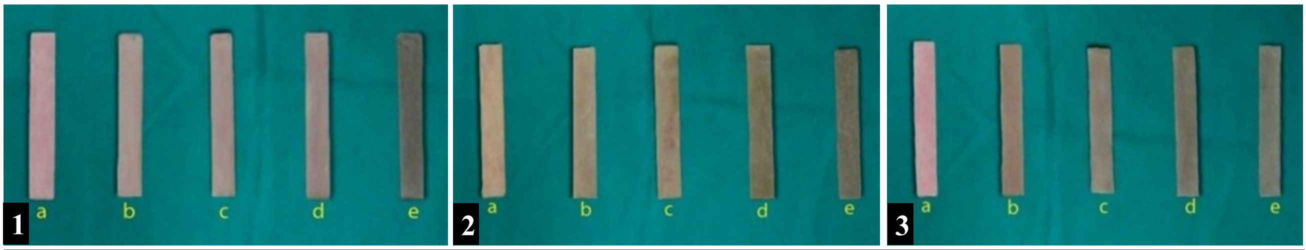
The materials used in the present study are detailed in Table 1.

2.1 Preparation of Acrylic Specimen

A total of 150 specimens were fabricated using the compression moulding technique. Acrylic specimens were made by investing rectangular metal strips of 62x10x2.5 mm. Metal strips were carefully removed after the investment material was set. A thin layer of separating medium was applied in the mould space and allowed to dry. Denture base acrylic powder with or without silver nanoparticles and monomer liquid was mixed as per the manufacturer recommendations and packed into the mould when the mix reached the dough consistency. Then the flask was closed, and a pressure of 4 lbs was applied and bench cured for 30 minutes in a hydraulic press (Silfradent, India). Then the flask was tightly secured in a clamp and transferred into a thermostatically controlled water bath (Acrylizer, Confident A-73, India) and cured as per the manufacturer's recommendations. The temperature of the water bath was increased to 73±1°C within 30 minutes and maintained at the same temperature for 90 minutes. Then the temperature of the water bath was increased to 100°C and maintained for 60 minutes. The flask was allowed to cool in the water

Table 1. Materials used in the study

Materials	Manufacturer's details
Trevlon	Dentsply India Pvt Ltd., India.
Lucitone 199	Dentsply International Inc., USA.
TriplexHot	IvoclarVivadent, USA.
Silver nanoparticles (80 – 100 nm)	Nanolabs Pvt Ltd., India.



Figures 1-3. Acrylic specimens made with Trevlon, Lucitone199 and TriplexHot denture base materials incorporated with Silver nanoparticles, respectively for evaluation of microhardness. Where a. Control (without nanoparticles), b. 0.5wt%, c. 1.0wt%, d. 2.0wt% and e. 5.0wt% of Silver nanoparticles .

bath to room temperature after completing the polymerization cycle. The acrylic resin specimens were retrieved after deflasking. The specimens obtained were finished and polished in a conventional manner [2]. Fifty specimens from each denture base material (Figures 1 – 3) were made totalling to 150 specimens. These 50 specimens from each denture base material were divided into five groups based on their concentration of silver nanoparticles (AgNPs), with ten specimens (n=10) in each group. These five groups included a control group (with no modification of denture base materials) and four modified groups with 0.5wt%, 1.0wt%, 2.0wt%, and 5.0wt%of AgNPs respectively. The specimens were stored in distilled water at 37^{0}C for seven days. .

2.2 Evaluation of Microhardness

Hardness was measured using Vicker's microhardness test apparatus, which has a diamond pyramid as indenter (Daksh Quality Systems Pvt Ltd., India). The test specimen was held firmly in position and lens were arranged to get the image clearly at its focal length. Subsequently, the indentation was made by applying the load of 25 gms for 25 seconds. A total of five indentations were made at different points for each specimen, and the mean values of individual specimens were averaged.

2.3 Scanning electron microscopy

The specimens were vacuum dried in a desiccator containing silica gel until a constant weight was obtained. The dried specimens were gold-sputtered and were subjected to scanning electron microscopy at 10 kV.

The data were subjected to One-way ANOVA and TukeyHSD tests for statistical analyses using SPSS for Windows, Version 12.0., SPSS Inc., USA.

3. Results

The concentration of the AgNPs was in direct proportion to the Vicker's hardness (HV) of three denture base materials used in the present study (Figure 4). Incorporation of 5.0 wt% of AgNPs showed maximum surface hardness. Among the three denture base materials, TriplexHot exhibited superior hardness at all the concentrations with the maximum mean hardness of 22.068 ± 0.321. Trevlon denture base material displayed less surface hardness at all concentrations compared to Lucitone 199 and TriplexHot materials. The control group of Lucitone199 specimens showed the least mean surface hardness (10.902 ± 0.390) among the materials tested. One-way ANOVA showed a significant difference (p = 0.000) in the surface hardness among the three groups.

PostHoc (Tukey's HSD) test showed significant differences (p=0.000) between unmodified and modified groups except with the Trevlon and TriplexHot groups modified by 0.5wt% of AgNPs (Table 2). Among the modified groups, significant differences were observed except between 2.0 wt% and 5.0 wt% of AgNPs in Lucitone199 denture base materials (Table 2).

PostHoc (Tukey's HSD) analysis showed significant differences (p = 0.000) among the three denture base materials incorporated with AgNPs, except Lucitone199 with Trevlon material at 0.5 wt%, and 1.0 wt% and also with TriplexHot material at 5.0 wt% (Table 3).

Scanning electron microscopic analysis (SEM) showed agglomeration of nanoparticles, and this agglomeration was found directly proportion to the concentration of nanoparticles in acrylic specimens (Figure 5).

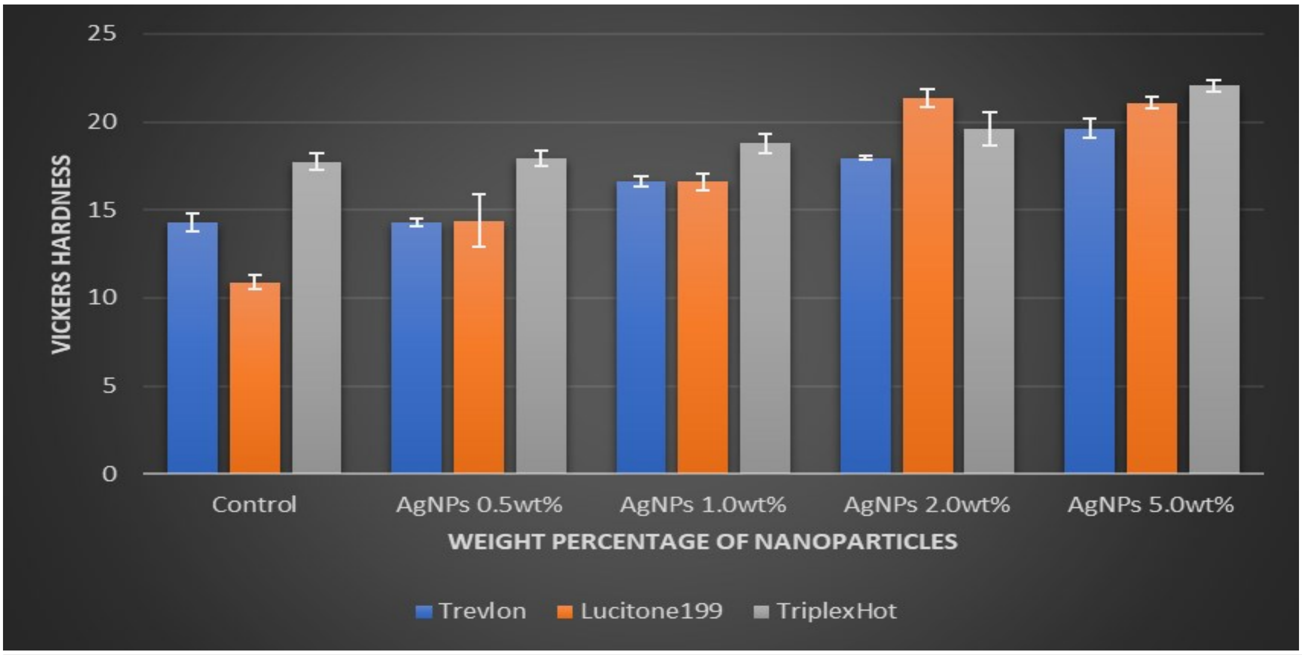


Figure 4. Vickers' hardness (HV) of denture base materials incorporated with various concentrations of Silver nanoparticles.

Table 2. Post-Hoc analysis (TukeyHSD) of Vickers' hardness (HV) of three denture base materials incorporated with various concentrations of Silver nanoparticles .

Groups		**Trevlon**		**Lucitone 199**		**TriplexHot**	
		Mean Diffeence ± Standard Error	Signifi-cance	Mean Diffeence ± Standard Error	Signifi-cance	Mean Diffeence ± Standard Error	Signifi-cance
Control	0.5 wt%	0.010 ± 0.165	1.000	3.493± 0.518	0.000	0.188± 0.258	0.949
	1.0 wt%	2.330±0.165	0.000	5.684± 0.518	0.000	1.053± 0.258	0.002
	2.0 wt%	7.078±0.165	0.000	10.463±0.518	0.000	1.868± 0.258	0.000
	5.0 wt%	11.082±0.165	0.000	10.789±0.518	0.000	4.340± 0.258	0.000
0.5 wt%	1.0 wt%	2.340±0.165	0.000	2.191± 0.518	0.001	0.865± 0.258	0.014
	2.0 wt%	3.683±0.165	0.000	6.970± 0.518	0.000	1.680± 0.258	0.000
	5.0 wt%	5.346±0.165	0.000	7.296± 0.518	0.000	4.152± 0.258	0.000
1.0 wt%	2.0 wt%	1.343±0.165	0.000	4.779± 0.518	0.000	0.815± 0.258	0.023
	5.0 wt%	3.006±0.165	0.000	5.105± 0.518	0.000	3.287± 0.258	0.000
2.0 wt%	5.0 wt%	1.663±0.165	0.000	0.326± 0.518	0.970	2.472± 0.258	0.000

Table 3. Post-Hoc analysis (TukeyHSD) of microhardness (HV) of denture base materials incorporated with various concentrations of AgNPs.

Groups			Significance (p-value)
Control	Lucitone199	Trevlon	0.000
		TriplexHot	0.000
	Trevlon	TriplexHot	0.000
0.5%	Lucitone199	Trevlon	0.954
		Triplex	0.000
	Trevlon	TriplexHot	0.000
1.0%	Lucitone199	Trevlon	0.987
		Triplex	0.000
	Trevlon	TriplexHot	0.000
2.0%	Lucitone199	Trevlon	0.000
		TriplexHot	0.000
	Trevlon	TriplexHot	0.000
5.0%	Lucitone199	Trevlon	0.002
		TriplexHot	0.761
	Trevlon	TriplexHot	0.000

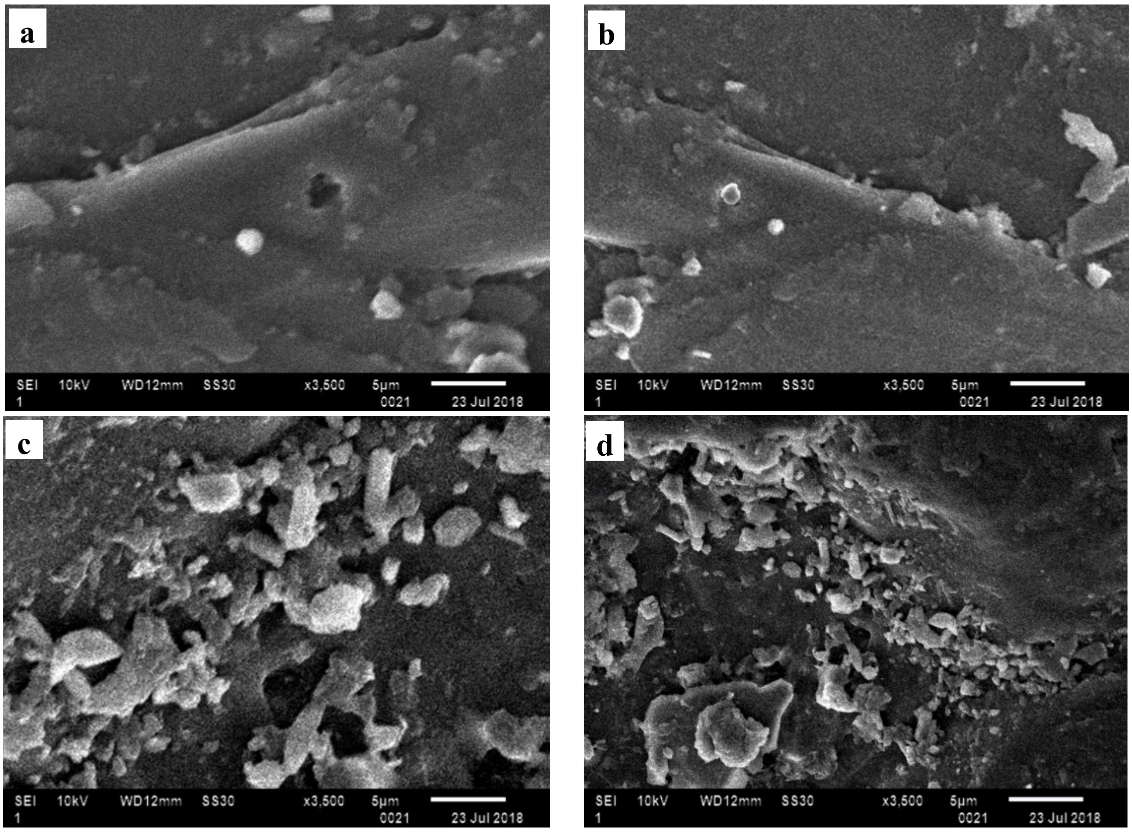


Figure 5. Dispersion of Silver nanoparticles in acrylic resin denture base material. Where a, b, c and d are denture base resin specimens incorporated with 0.5wt%, 1.0wt%, 2.0wt% and 5.0wt% of Silver nanoparticles respectively.

4. Discussion

An ideal denture base prosthesis must have maximum resistance to abrasion under a variety of loads. Conventional cleansing agents may soften the denture base materials leading to fracture [11-13]. Unfortunately, PMMA-based denture base materials are weak at resisting abrasion, which may create surface irregularities on the prosthesis. These irregularities can become the areas of debris accumulation and cause stomatitis [10]; hence, it is necessary to enhance the hardness of an acrylic resin. Numerous researchers have suggested that incorporation of AgNPs serves as good antimicrobial agents in denture base materials and denture lining materials, and may be considered as alternatives to the regularly used antibacterial and antifungal drugs [8,14-16]. This study evaluated the effect of AgNPs incorporation on surface hardness of denture base materials using the Vickers hardness test. The hardness was tested using the ability of the material to resist the indentation of a specific load [1,17].

The degree of conversion of heat-cured acrylic resin may be evaluated indirectly by measuring the surface hardness[18]. There are many factors; the matrix composition, filler content, filler shape, size distribution, and proportion having a maximum effect on the mechanical and physical properties of reinforced resin [19,20].

In the present study, it was observed that the hardness was directly proportional to the concentration of nanoparticles incorporated. Both unmodified and modified groups of TriplexHot denture base material exhibited more Vickers hardness among the materials tested. A gradual increase in hardness was observed in all the modified groups with an increase in the weight percentage of nanoparticles. One-way ANOVA showed a significant difference ($p = 0.000$) in hardness among the three denture base materials. The hardness value was increased by increasing the concentration of nanoparticles (Figure 1). It is because of the nanoparticles' surface area to make suitable adhesive with the polymer. Therefore, the size of the nanoparticles used in this study may be considered as hard fillers in the resin matrix. The larger particles may form larger voids in the resin matrix, and these act as stress concentrators. In the present study, the average particle size used was in the range of 80–100 nm. However, it was also evident from the literature that nanoparticles agglomerate at higher concentrations and attaches physically with the PMMA polymer chains. These physically bonded nanoparticles may be separated easily during indentation and facilitate the easy crack propagation through the resin matrix and results in a fracture. The SEM evaluation (Figure 5) showed the agglomeration of the nanoparticles in this study but had not shown any effect on the hardness. Numerous SEM studies indicated similar findings during microhardness testing though agglomerates were formed [21]. Besides, these nanoparticle agglomerates form a thick immobilized PMMA layer, which resists indentation. This phenomenon is evident from this study that the Vicker's hardness was more at 5.0 wt% of AgNPs incorporation in all the denture base materials.

The results of this study are in agreement with Vojdani *et al.* (2012) [22] and Masouras *et al.* (2008) [23]. According to them, the surface hardness improves with increasing the concentration of filler particles; thus, the filler amount contributes to the material performance. These results are also similar to Sodagar *et al.* (2013) [24], who showed that the effect of silver filler particle size on the internal structure of polymerized PMMA. Similarly, Sokolowski J *et al.* (2014) [25] also reported an increase in Vicker's hardness of resin adhesives by the increase in silver nanoparticle concentration. They also suggested that the incorporation of silver nanoparticles may not have any adverse effect on mechanical properties of composite adhesives containing silica nanofillers and silver nanoparticles if proper amounts of silver nanoparticles are used.

The other factor which influences the hardness is the aging of the specimens. Several studies reported that aging results in an increase in hardness of denture base materials modified with various nanoparticles [26], as it facilitates more degree of polymerization. In this study, the specimens were also soaked in distilled water for seven days before the hardness test was carried out. Fan *et al.* (2011) [27] demonstrated that in terms of Rockwell hardness measurements, the degree of cure was lowered as the concentration of silver benzoate was increased in light-cure composite resins. The authors suggested that Ag^+ ion reduction and the generation of clusters of atoms and nanoparticles while curing causes competition with the free radical polymerization process. However, the resin materials used in this study were heat-cured resins in which free -radical addition polymerization was unaffected though the nanoparticles agglomerated. Also, aging the specimens caused an increase in microhardness [28].

On the contrary, Alhareb *et al.* (2017) [29] reported less Vickers hardness with the incorporation of the nanoparticles and nitrobutyl rubber. The reason for the decrease in hardness can be attributed to nitrobutyl rubber that provided more ductility to the resin matrix and had a detrimental effect on hardness. Similarly, Asopa V *et al.* (2015) [30] also reported a decrease in hardness by the incorporation of nanoparticles attributing it to a lack of bonding between the resin matrix and the nanofillers. Chladek *et al.* (2013) [31] reported that the hardness is increased as the nanoparticles concentration increased to a certain level beyond which a decrease in hardness was found. So, it can be attributed that more the concentration of nanoparticles there is a possibility for more agglomeration and creates more or large voids at the filler–resin interface which results in a decrease in hardness and strength. However, the present study reports an increase in the hardness with a maximum concentration of nanoparticles (5.0 wt%) that was used for the three denture base materials.

The variation in the hardness of three denture base materials with and without the addition of nanoparticles can also be attributed to the chemical composition of the denture base resins, mostly the amount of the crosslinking agent and plasticizer [32] that were present in the individual denture base materials. However, incorporation of 5.0wt% of NPs significantly improved the Vickers hardness among all the denture base materials tested.

5. Conclusion

From this study, it can be concluded that the incorporation of AgNPs increases the surface hardness of denture base materials used in the study. Since the silver nanoparticles already proved their antimicrobial characteristics, their incorporation into denture base materials may also be considered to enhance the surface hardness. However, incorporation of AgNPs in higher concentrations impart black colour to the denture bases, which is undesirable. Further studies are required to address the biocompatibility issues and the colour stability of denture base materials incorporated with the silver nanoparticles.

Conflicts of interest: Authors declared no conflicts of interest.

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