# Comparative Evaluation of the Flexural Strength of Acrylic Denture Base Resin Modified with Different Concentrations of Colloidal Silver Nanoparticle's subjected to Short Curing Cycle – An *In vitro* Study

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## Abstract

**Objective:** The objective of the study was to analyze the flexural strength (FS) of polymethyl methacrylic resin processed with short curing cycle after incorporating 0%, 0.5%, and 5% concentrations of silver nanoparticle's as antimicrobial agent. **Materials and Methods:** The use of universal testing machine (Instron, Model No. 5967) by collecting the FS values from specimens for analytical interpretation in three subgroups with incorporation of 0%, 0.5%, and 5% concentration of colloidal silver nanoparticle's cured with short curing cycle. Microsoft Excel Software (SPSS for Windows Version-20) (2010) helped to evaluate the absolute count of FS parameters of each specimen of three subgroups and to summarize data into the forms of tables. **Results:** In comparison with the three subgroups, the mean FS of modified denture base resin by adding silver nanoparticles was gradually decreased at concentrations (0%, 0.5%, and 5%). At short curing cycle, modified denture base resin showed lower FS than unmodified resin. **Conclusion:** The method of curing process proved that short curing cycle is good and time saving process due to uses of terminal boiling temperature which cause/produce fast and complete polymerization reaction for curing the denture base resin.

**Keywords:** Denture stomatitis, Flexural strength, Polymethyl methacrylate, Silver nanoparticle's.

## Introduction

The use of a complete denture is necessary for functional and esthetic rehabilitation of edentulous mainly geriatric or hospitalized patients due to cognitive impairment, reduced motor dexterity, and memory loss.[1,2] Denture stomatitis (DS) is the most common inflammatory condition which causes by *Candida albicans* that affects edentulous patients who wear the dentures. DS is characterized by generalized inflammation or reddening of the palatal mucosa underneath the denture. PMMA is most widely used resin material for denture fabrication.[3-6] A method of treatment by combining tissue conditioner and antifungal agents was suggested by Douglas and Walker.[5] Silver has received a great deal of attention as antimicrobial agent in history but it also effects on mechanical properties of the resin.[4,6]

There is a very few studies about addition of AgNPs to denture base resins for sanitation and their influences over properties to denture base resins have been published.

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In the present study, 0.5% and 5% concentrations of AgNPs are incorporated in PMMA in order to minimize the probable unfavorable changes on mechanical and chemical properties of denture acrylic base.

Thus, the purpose of the present study is to evaluate the flexural strength (FS) of heat-cured denture base acrylic resin modified with different concentrations of colloidal AgNPs subjected to short curing cycle.

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## Materials and Methods

One commonly used commercial brand of heat-cured acrylic resin Lucitone-199 PMMA (Dentsply York Division, USA) denture base resin and 10 nm size of colloidal silver nanoparticle's with 40 ppm concentration (Reinste Nano Ventures Pvt. Ltd.) were used in this study. Source of data for evaluating FS was collected from specimens prepared using conventional heat cure PMMA denture base resin modified with 0%, 0.5%, and 5% concentrations by volume colloidal silver nanoparticle's.

A total of 30 specimens were prepared, which were further divided in three subgroups A1, A2, and A3 with 10 specimens in each subgroup prepared by incorporation of 0%, 0.5%, and 5% concentrations by volume of colloidal silver nanoparticle's subjected to short curing cycle at 74° C for 1½ h and 100° C for ½ h by the following procedure.

The bar-shaped metal (steel) pattern was constructed with specific dimension of 65 mm × 10 mm × 2.5 mm (Figures 1 and 2) length, width, and thickness, respectively, ass per ADA specification No. 12. The accuracy of the dimensions was verified with a micrometer at three locations for each dimension to within a 0.05 mm tolerance for width, height, and thickness. The steel dies were invested with dental plaster (Neelkanth Dental Plaster Class-2 Pvt. Ltd., Mumbai, India) in Hanau flask (Hanau Eng. Co. Buffalo, NY, USA) (Figure 3) using conventional compressive technique. The dental plaster in the shallow half of the flask was poured using electrical vibrator against a glass plate to obtain smooth surface. Two patterns were invested in each flask. Dewaxing was done. The flask segments were separated and the steel dies were removed using air pressure, after the flask get room temperature. A thin, single uniform layer of separating medium (Cold Mold Seal Dental Product of India) was applied to dental stone on both the parts of the flask. The aqueous colloidal dispersion containing colloidal silver nanoparticle's was added into the monomer of the acrylic resin at 0%, 0.5%, and 5% concentrations based polymer mass, weighed by an accurate digital weighing machine. PMMA resin polymer was mixed in a clean dry jar (as per manufactures instructions, P/L ratio 3:1 by volume). The mixture left covered until dough stage. The mixture was removed from the jar and packed into stone mold, which was previously coated with separating medium. The two halves of the flask were closed together and placed under hydraulic pressure, which was slowly applied on the flask to get flow of the resin dough throughout the mold space. Finally, the halves of flask were closed together metal to metal and held for 5 min before clamping than transferred to water bath. Then, flasks were placed for curing the specimens in acrylizer for short curing at 74°C for 1½ h and at 100°C for ½ h to make 30 specimens. Then, the flask was left to cool slowly for 30 min before deflasking and the specimens were retrieved from the mold.

### Finishing and polishing

All flasks of acrylic were removed with an acrylic bur to get a smooth surface. The acrylic trimming bur should be used followed by 120 grain size sandpaper to remove any remaining small scratches with continuous water cooling. Polishing was accomplished using bristle brush and pumice with lathe polishing machine. A glossy surface was obtained with wool brush and polishing soap on dental lathe using low speed (1500 rpm) and the specimens were continuously cooled with water to avoid overheating, which may lead to distortions of the specimens. Then, the final measurements of the specimens were obtained using the wax gauge. After finishing polishing (Figures 4a-c), the specimens were measured again to ensure exact measurement.

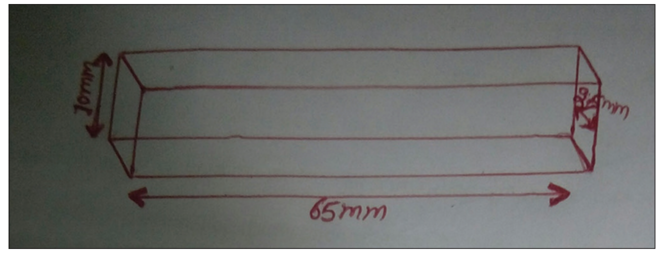



**Figure 1:** Schematic representation of die dimension

**Figure 2:** Metal dies used to create mold space

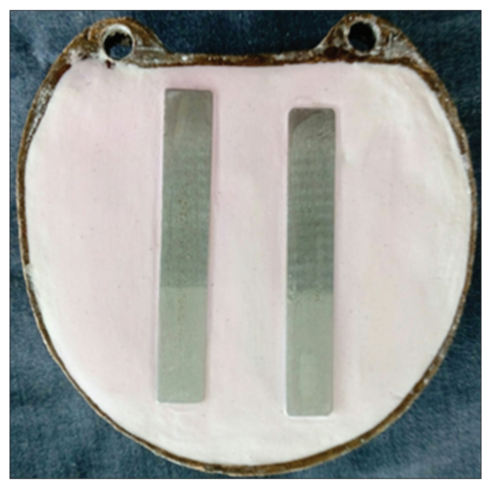


**Figure 3:** Invested dies to create mold space

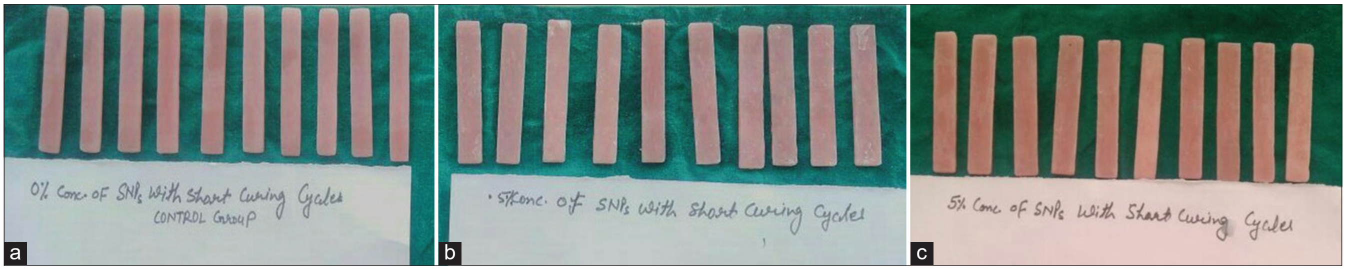



**Figure 4:** (a-c) Finished polished specimens

## Indentation of flexural test

The FS of all specimens was tested under universal testing machine (Instron, Model No. 5967) (Figures 5 and 6). Three-pointed indenter 0.8 mm in diameter which penetrated the material surface by pressure applied on the tester down firmly and gradually on the indenter and records the maximum reading until specimen break down. For the detection of FS, the UTM was set at a crosshead speed of 2 mm/min. A load was applied using a centrally located rod until a fracture occurs. Data of FS were collected from each specimen, taken directly from the digital screen reading (Table 1). The data were entered and statistical analysis was done using Statistical Package for the Social Sciences (SPSS Version 20; Chicago Inc., USA). The quantitative data were presented in the form of mean and standard deviation and qualitative data in terms of frequencies and percentages. Relevant statistical tests were used, that is, analysis of variance (ANOVA), Tukey's *post hoc* analysis, and unpaired Student's "t"-test (Table 2).

## FS testing

The FS was carried out on the universal testing machine. All the specimens were tested using three-point bending test with a crosshead speed of 2 mm/min. FS was determined using the following formula:

$FS = 3Fl/2bh^2$

Where, F is the maximum load applied (N), I is the distance between supports (span length=50 mm), b is the width of the specimens (10), and h is the thickness of the specimen (2.5 mm).

## Statistical analysis

In each subgroup, the quantitative data were calculated in the mean and standard deviation and qualitative data were calculated in terms of frequencies and percentages (Table 2). For statistical analysis, relevant statistical tests were used, that is, analysis of variance (ANOVA), Tukey's *post hoc* analysis, and unpaired Student's "t" test.

# Results

It was analysed that denture base resin with 0% concentration of Ag nano particles showed maximum flexural strength (3631.90 MPa) than at 0.5% concentration. The flexural strength at 5% concentration of Ag nano particles is 2491.90 MPa. FS values were decreased gradually as concentration of AgNPs increased


**Figure 5:** Universal testing machine

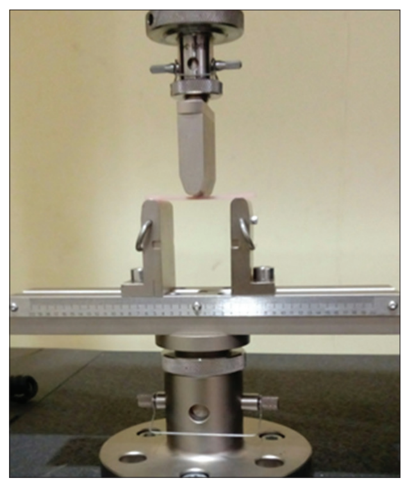


**Figure 6:** Specimen subjected to loading in the universal testing machine

(Graph 1) because AgNPs act like a plasticizer and decrease FS of denture base resin.

# Discussion

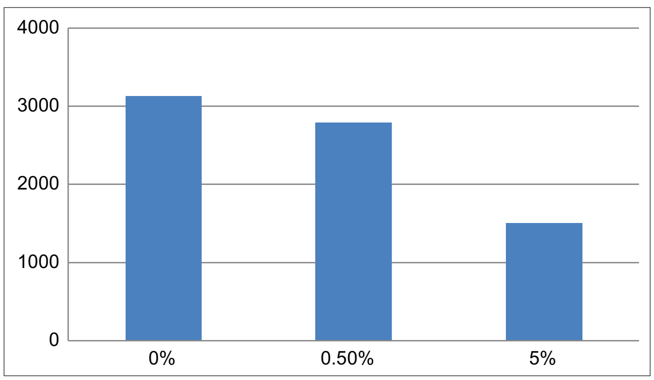
Denture bases can be fabricated using various materials, including metals and heat-cured acrylic resins. Metal denture base is not preferred due to several disadvantages and difficulty in tissue replacement in severely resorbed alveolar ridges and inability to reline. Acrylic resins have been used widely because of their good esthetics and favorable characteristics.

**Table 1: UTM data of flex modulus of each subgroup specimens**

| S. No. | Subgroup-A1-0% (control group) MPa | Subgroup-B2-0.5% MPa | Subgroup-B3-5% MPa |
|---|---|---|---|
| 1. | 3607.34 | 3036.83 | 2555.85 |
| 2. | 3654.26 | 3057.08 | 2489.04 |
| 3. | 3627.71 | 3011.55 | 2405.35 |
| 4. | 3684.17 | 2958.10 | 2456.38 |
| 5. | 3672.61 | 2956.55 | 2431.46 |
| 6. | 3581.35 | 2967.70 | 2502.92 |
| 7. | 3677.06 | 3056.21 | 2568.3 |
| 8. | 3623.57 | 3032.09 | 2566.62 |
| 9. | 3592.95 | 3023.35 | 2515.75 |
| 10. | 3598.07 | 3067.29 | 2427.42 |

**Table 2: Mean and standard deviation of flexural strength**

| Group-A | N | Mean | SD |
|---|---|---|---|
| A-1, 0% | 10 | 3631.909 | 37.76516 |
| A-2, 0.5% | 10 | 3016.675 | 42.05383 |
| A-3, 5% | 10 | 2491.909 | 60.34283 |
| F | | 1428.829 | |
| *P* | | <0.001 | |




**Graph 1:** Mean and standard deviation of flexural strength

These materials account for approximately 95% of the denture base materials used in prosthodontics. The majority of prosthetic acrylic resins consist of excellent esthetic properties, sufficient strength, low water sorption, and low solubility. They are very accurate in reproducing surface detail and can be easily repaired. There are few important drawbacks which are inherent in these resins, such as low thermal conductivity, high coefficient of thermal expansion that causes internal stresses to be released during the processing resulting in dimensional inaccuracy, and relatively low modulus of elasticity which causes its rapid deformation at low stresses.[7] Furthermore, one of the disadvantages of acrylic resin is that the tissue surface of denture base is susceptible to *Candida albicans*. Hence making it as effective reservoir of microorganism. The presence of cracks or porosities in the denture acrylic resins acts as fungi reservoirs. Candidiasis is the most common fungal infection that occurs in the mouth and it is characterized by homogeneous erythema or red focal areas, especially in the palatal mucosa. Immune defense of the host and local defense responses are actively involved in the resistance to the fungus pathogenic mechanisms, development, and progress of infection.[8]

The treatment of DS is problematic since recurrent infection is frequently encountered after the end of treatment. Studies have shown that antifungal drug resistance hinders the successful clinical treatment of candidiasis in denture wearers. Therefore, it is prudent to evaluate alternative materials which may hinder *Candida* adherence. The methods for the prevention of DS, such as the incorporation of antimicrobial agents into polymers used as denture base and as tissue conditioners, have been developed. In contrast, silver has been widely used in medical and life care in the form of polymers and colloidal which exhibits antimicrobial action against Gram-positive and Gram-negative bacteria and fungi. Silver ions bind to electron donor groups in biological molecules containing sulfur or nitrogen, resulting in preventing cell reproduction.[9]

The antimicrobial properties of silver, especially when the NPs are added to the denture, have been reported in several studies. Chladek *et al.* showed that AgNPs inhibit the fungus *Candida albicans* growth and adding this material to the dentures could reduce oral diseases among edentulous patients.[10] Catalytic oxidation by metallic silver and reaction with dissolved monovalent silver ion contributes to its bactericidal effect. Microbes are unlikely to develop resistance against silver, as they do against conventional and narrow target antibiotics because the metal attacks a broad range of targets in the organisms.[11]

Thus, in the present study, AgNPs are used as an antimicrobial component in PMMA resin.

The use of AgNPs as antimicrobial agents has received a great deal of attention, due to their small size. Nanoparticles provide a large surface area for oxidation. The concentration higher than 10% of the AgNPs had toxic effects on epithelial cells and at lower concentration did not have significant effect on the viability of cells. Therefore, in the present study, 0.5% and 5% concentrations of AgNPs are incorporated in PMMA resin, which do not have significant effect on the viability of cells and minimize the probable unfavorable changes in mechanical and chemical properties of denture base acrylic resin.

In the 2011, Fan *et al.* confirmed that there is a change in mechanical characteristics of the modified materials by increasing the AgNPs concentrations.[12] Thus, there are concerns regarding the physical properties of these modified polymers. Zappini *et al.* not noted that FS is important because it reflects the rigidity of material. For this reason, flexural bend test was selected as it was most relevant to clinical conditions of the masticatory load to evaluate the strength of acrylic resins.[13] Thus, the purpose of the present study was to evaluate FS of PMMA resin processed with long curing cycle after incorporating 0.5% and 5% concentrations of AgNPs as antimicrobial agent.

Heat curing is the most popular method of processing denture base acrylic resin. The heat curing system was selected due to its adequate accuracy, good resistance to attack by solvents and laboratory cost is substantially less.

For the preparation of modified PMMA resin with AgNPs, two approaches have been developed: (a) Mixing nanoparticle's with polymer and (b) adding nanoparticle's into the monomer.

In the present study, the method for adding NPs into acrylic resin material was in the form of liquid added to the monomer of acrylic resin. This method can be explained, as the distribution of particles is more equal than if placed in the powder form of AgNPs into acrylic powder. This method also reduces the agglomeration. The prepared aqueous solution of AgNPs was dispersed in acrylic liquid in the desired ratio and then mixed with powder part of acrylic material.

The acrylic resin test specimens were prepared according to the ADA specification No. 12 for heat-cured denture base acrylic resin to standardize the dimension for all the specimens for testing procedures. The acrylic resins specimens used in this study were prepared in thickness of 2.5 ± 0.5 mm as the actual average thickness of denture base. A metal die was used to standardize the dimensions for all the specimens. The testing machine Instron-5967 was chosen due to its technical specification.

The present study was conducted to determine the effects of AgNPs on FS of PMMA resin modified with 0.5% and 5% concentrations cured with long curing cycle.

The result of this present study showed that in specimens of modified PMMA resin cured with long curing cycle flexural strength decreases as silver nanoparticle concentration increases. A statistically significant decrease of FS in comparison to the control group was observed as increasing AgNPs concentration in the resin specimens.

These results are supported by the finding of the study conducted by Sodagar *et al.* They reported that the addition of 0.05% concentration AgNPs in rapid repair resin decreased FS significantly. It has been reported that FS of one brand of resin decreased and in other brand of resin FS increased after incorporation of AgNPs. Hence, the type of acrylic resin and the amount of nanoparticle's incorporated are the important factors which affect the strength of PMMA resin.

The result of this study indicated that incorporation of AgNPs significantly decreased the FS of PMMA resin, which might be due to less dispersion of NP's per unit area of PMMA resin matrix. In the present study, size of nanoparticle's used was 10 nm. This also enhances the chances of void formation from entrapped air, moisture, and incomplete wetting of the nanoparticle's by resin.

Incorporation of nanoparticle's causes resin particles to agglomerate and aggregate. The agglomerated compounds can act as stress concentrating centers in the matrix and adversely affect mechanical properties of the polymerized material. There is one more disadvantage in incorporating nanoparticle into acrylic resin that is lack of chemical bond between inorganic materials such as AgNPs and PMMA resin. To improve bonding between metal and resin, some chemicals such as 4-methacryloxyethyl trimellitate anhydride and G-methacryloxypropyltrimethoxysilane have been used. In the present study chemical interaction between PMMA resin and silver nanoparticle is low that causes decrease in flexural strength.

Furthermore, the finding of this study was in agreement with a study conducted by Korgolu *et al.* They concluded that the addition of 0.8 wt% and 1.6 wt% concentrations of AgNPs alters the FS and elastic modulus of microwave-cured PMMA resin. This may have resulted from the AgNPs ratio acting as impurities within the resin, which leads to decrease in mechanical strength of the resin.

In contrast to our study, the study conducted by Kassee *et al.* indicated that adding 0.5% concentration AgNPs into self-curing acrylic resin system increases the FS. The differences in acrylic resin type, size of AgNPs, amount of nanoparticle's, and polar attractions which formed between c = o groups of the polymethyl methacrylic chain and silver nanoparticle's may be responsible for this situation.

In the current study, 0%, 0.5%, and 5% concentrations of AgNPs were used. By utilizing low concentrations of nanoparticle's, material costs and the amount of monomer used can be reduced, thus rendering our process cost effective.

Ghafferi *et al.* in a study reported that AgNPs with 0.2 wt% and 2 wt% concentrations increased the compressive strength of acrylic resin, but increasing AgNPs concentration from 0.2 wt% to 2 wt% concentrations did not improve FS of acrylic resin significantly. Tensile strength results showed different behavior patterns and incorporation of AgNPs decreased tensile strengths in both groups containing AgNPs because in tensile strength was not significant by addition of 0.2 wt% concentration of colloidal silver nanoparticle's, whereas high concentration of 2 wt% concentration exhibited significantly low tensile strength. Silver nanoparticles act as impurities and tensile strength would decrease by increasing this content. The acrylic resin with AgNPs is more brittle than pure resin because of low-energy absorption during fracture. Furthermore, due to the addition of nanoparticle's, the un-reacted monomer quality may be increased depending on the decrease in the monomer reaction and it acts like a plasticizer.

The data of the present study show with modified PMMA resin cured with long curing cycle which has lower FS. The data of the present study are in agreement with the study conducted by Jerolimov *et al.* who stated that the choice of curing cycle has much greater influence on the level of residual monomer as compared to the mixing ratio.[14]

Bayrallar *et al.* found that short curing cycle without post-polymerization terminal boil showed higher content of residual monomer as compared to a shorter cycle followed by a short terminal boil. Hence to reduce the final residual monomer content, a final post-polymerization treatment is more effective.

In the present study, Group B specimens underwent a terminal boil post-polymerization treatment, which could be the reason for low residual monomer content and less reduction in FS as compared to subgroups specimens. As the polymerization reaction proceeds, the amount of monomer and free radicals decreases and due to increase in viscosity and degree of polymerization, it becomes difficult to bring the monomer and free radical together. Hence, the diffusion of monomer becomes the rate-limiting step. Increasing the temperature causes the molecules to move faster and the polymerization reaction will be more complete.

According to Harison and Hugget, the long water bath cycle without terminal boil shows levels of residual monomer approximately 3 times greater than when a terminal boil is used. The residual monomer could affect the FS because of a plasticizing effect.[15] The terminal boil was not used in the long curing cycle in the present study.

As described earlier, reasons for reduced FS of long curing cycle without terminal boil could be due to incomplete decomposition of initiator benzoyl peroxide at slow temperature polymerization is time dependent and the rate of conversion is greatly measured by increasing the temperature from 74° C to 100° C. However, it is important to realize the FS values obtained for the specimens are above minimum values (65 MPa) set forth by the ADA specification No. 12 and it may have no clinical relevance.[16]

The present study has its limitations because it was done under laboratory conditions. No method to simulate mouth temperature and no moisture equivalent to saliva were used. There was no way to simulate the biofilm that exists on oral surfaces and come into contact with the denture base material. There were no uses of any UV spectrophotometer to determine the amount of monomer released into distilled water. The toxicity of residual monomer was also not detected. The properties of denture base in the clinical situation still differ from the laboratory testing conditions.

Further investigations should incorporate more closely simulated clinical conditions. There is also lack of chemical bond between AgNPs and PMMA resin methods can be extrapolated to find out appropriate substances as coupling agents between AgNPs and PMMA resin. Therefore, it might be possible to decrease its deleterious effects on mechanical properties.

Furthermore, in the present study, Lucitone 199 denture base material FS was tested with incorporation of different concentrations of AgNPs (0%, 0.5%, and 5%) subjected to long curing cycles. Difference in the curing methods of denture base materials could affect the variations present in result. These factors also need further investigation to identify and predict which materials, polymerization methods, and which adequate concentration of silver nanoparticle's will provide the best service clinically.

## Clinical significance

The major advantages of the addition of AgNPs to PMMA resin could be for elderly people with restricted manual dexterity or cognitive disturbances, especially for patients who do not follow an adequate denture cleaning protocol and diabetic patients who are more susceptible for DS.

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