



## RESEARCH ARTICLE

### EFFECT OF NANO CLAY AND SILVER NANO PARTICLES ON THE FLEXURAL STRENGTH AND ANTIMICROBIAL PROPERTY OF ACRYLIC DENTURE BASE RESIN- AN INVITRO STUDY

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#### ABSTRACT

Polymethyl methacrylate (PMMA) has been commonly used as denture base material. However, repeated stress from masticatory action during function and impact force from accidental drop can result in fracture of the denture base. Thus, further improvements in the mechanical property of PMMA denture base material are still needed. This study aimed to provide us with an improved acrylic denture base resin which possesses better mechanical property and antimicrobial activity by addition of nano clay and silver nano particles. The objectives of the study are to evaluate the flexural strength and antimicrobial property of conventional PMMA denture base resin after incorporation of 3% nano clay and varied concentrations of silver nano particles.

**Materials and Methods:** A total number of 60 specimens were included in the study. 30 rectangular specimens were fabricated for testing the flexural strength and 30 disc shaped specimens were fabricated for testing the antimicrobial property. Test specimens were divided into 3 groups based on the concentration of nano clay and silver nano particles in heat cure acrylic resin. These included a control group and two test groups with 3% nano clay and varied concentrations of silver nano particles.

**Results:** One way ANOVA descriptive analysis showed significant difference after the incorporation of 3% nano clay and different percentage of silver nano particles in conventional PMMA denture base resin. The increasing order of flexural strength was as follows: Group B (3% nano clay and 0.5% silver nano particles) showed the least flexural strength followed by Group C (3% nano clay and 1% silver nano particles) and Group A (Control) showed the highest flexural strength. The increasing order of antimicrobial property is as follows: Group A (Control) showed the least antimicrobial property followed by Group B (3% nano clay and 0.5% silver nano particles) and Group C (3% nano clay and 1% silver nano particles) showed the highest antimicrobial property.

**Conclusion:** The flexural strength of conventional PMMA denture base resin after incorporation of 3% nano clay and 0.5% silver nano particles showed the least flexural strength. Incorporation of 3% nano clay and 1% silver nano particles showed higher flexural strength but lesser compared to the control group. The antimicrobial property of conventional PMMA denture base resin after incorporation of 3% nano clay and 0.5% silver nano particles showed lower antimicrobial property but higher compared to the control group. Incorporation of 3% nano clay and 1% silver nano particles showed the highest antimicrobial property.

**Clinical implications:** This study provided a feasible approach of developing a modified acrylic denture base resin having sufficient flexural strength and possessing antimicrobial properties. The optimum microbial inhibition shown by the synthesized material can be considered to be an interesting material for scientific research of antimicrobial acrylic denture base resins.

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## INTRODUCTION

Dentures are made of poly methyl methacrylate (PMMA) acrylic resin.<sup>1</sup> PMMA acrylic resin has proven to be the most satisfactory denture base material currently available. Despite their popularity, this material is far from ideal in fulfilling the mechanical requirements. Recently the use of nanoparticles as fillers or additives in polymers for various desired effects are receiving an increased interest for research and development.

Various types of nanoparticles, including nanocarbon, carbon nanotubes, nanoclays, and metal oxides, are currently used to modify the polymer's performance.<sup>2</sup> Flexural strength is a critical parameter for selecting denture base material, as the denture bases must endure various types of stress during function. Decreased flexural strength of the denture base resin will lead to its fracture when subjected to flexural stress. Polymer nanocomposites exhibit excellent biocompatibility as denture base material and have potential application in dental materials.<sup>3</sup> Nano clay dispersed into the PMMA matrix improves the structure, flexural strength and thermal stability

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of PMMA,<sup>4</sup> and may decrease the possibility of fracture of the denture base resins. As nano clay is organic in nature, chances of microbial colonization may increase. To compensate for this, incorporation of an antibiotic agent is necessary. As the denture base resin is subjected to heat curing cycles, finishing and polishing procedures which generate huge amount of heat, it may lead to instability of the antibiotic agent within the acrylic denture base resin. Hence we require an antimicrobial agent that overcomes this drawback and is stable within the PMMA matrix when incorporated into the acrylic denture base resin. Silver (Ag) has been well known for its antimicrobial characteristics and has a long history of its application in medicine with a well-tolerated tissue response and low toxicity profile.<sup>5</sup> Due to their small size, nano particles present a large surface area and therefore larger available area for oxidation.<sup>6</sup> These silver nano particles can be used to overcome the drawbacks of an antibiotic agent and also are stable within PMMA matrix of the acrylic denture base resin.

Hence addition of nano clay and silver nano particles may provide us with an improved acrylic denture base resin which possesses better mechanical property and antimicrobial activity. Chronic atrophic Candidiasis, also known as denture stomatitis is a common form of oral Candidiasis involving oral mucosal tissues in complete denture wearers, associated with the adherence of *Candida albicans* to denture base surfaces.<sup>7</sup> Oral mucosal infection with *Staphylococcus aureus* has recently been incriminated in a severe form of mucositis in some group of elderly patients with systemic diseases.<sup>8</sup> In the present study, antimicrobial activity against *Candida Albicans* and *Staphylococcus Aureus* were evaluated. The purpose of this in-vitro study was to evaluate the flexural strength and antimicrobial property of conventional PMMA denture base resin after incorporating 3% nano clay and varied concentrations of silver nano particles.

## MATERIALS AND METHODS

### Fabrication of specimens for assessment of flexural strength and antimicrobial property

A total number of 60 specimens were included in the study. 30 rectangular specimens were fabricated for testing the flexural strength and 30 disc shaped specimens were fabricated for testing the antimicrobial property against test strains of *Candida albicans* and *Staphylococcus aureus*. Test specimens were divided into 3 groups based on the concentration of nano clay and silver nano particles in heat cure acrylic resin.

**Group A:** Heat cure acrylic resin with 0% nano clay and silver nano particles (control)

**Group B:** Heat cure acrylic resin with 3% nano clay and 0.5% silver nano particles (by Wt)

**Group C:** Heat cure acrylic resin with 3% nano clay and 1% silver nano particles (by Wt)

Uniform size wax patterns (65×10×2.5 mm rectangle shaped for flexural strength and 6×1 mm disc shaped for antimicrobial property) were fabricated using standardized stainless steel mould for making acrylic resin test specimens to test flexural strength and antimicrobial property.

### Preparations of heat cure acrylic resin without Nano clay and Silver nano particles (control)

DPI heat cure denture base resin was mixed in a ceramic mixing jar according to the manufacturer's recommended ratio of polymer and monomer (3:1 by volume). The resin was left in the mixing jar until it reached the dough stage, and then the mix was kneaded thoroughly to obtain a homogenous dough. The dough was packed into the molds with a thin cellophane sheet placed over it. The flask lid was closed properly and was placed in the hydraulic bench press. The pressure was gradually raised up to 1500 psi pressure to compress the material into the mold. Trial closure was carried out, till no flash was present. The cellophane sheet was removed and the final closure was done. The flasks were bench cured for 1/2 hour at room temperature.

### Preparations of heat cure acrylic resin with Nano clay and Silver nano particles

Materials were weighed in an analytical balance, nano clay in (Sigma Aldrich, Nanomer® PGV) and followed by silver nano particles (Ag Flex Technologies Private Ltd, Flexsil™) were incorporated in pre-defined percentages to the polymer powder of the denture base acrylic resin. These were mixed using an ultrasonic stirrer to obtain a uniform particle distribution. This mixing of nano clay and silver nano particles with acrylic resin polymer resulted in even distribution of filler within polymer matrix. Denture base resin was mixed in a ceramic mixing jar according to the manufacturer recommended ratio of polymer and monomer (3:1 by volume). The resin was left in the mixing jar until it reached the dough stage, and then the mix was kneaded thoroughly to obtain homogenous dough. The dough was packed into the molds with a thin cellophane sheet placed over it. The flask lid was closed properly and was placed in the hydraulic bench press. The pressure was gradually raised up to 1500 psi pressure to compress the material into the mold. Trial closure was carried out, till no flash was present. The cellophane sheet was removed and the final closure was done. The flasks were bench cured for 1/2 hour at room temperature.

After processing, the specimens for testing flexural strength were retrieved, finished, marked and stored in distilled water at 37°C ± 1°C for 48 hrs prior to subjecting them to flexural strength testing. After processing, the specimens for testing antimicrobial property were retrieved, finished, marked, autoclaved and stored in artificial saliva at 37°C ± 1°C for 7 days prior to subjecting them to antimicrobial property testing.

### Assessment of Flexural Strength

The flexural strength of the specimens was determined using a 3-point bend testing device in a Lloyd's Universal Testing Machine at Jaycee engineering college. The device consisted of a loading wedge and a pair of adjustable supporting wedges placed at 50mm apart. The specimen was centred on the device in such a way that the loading wedge, set to travel at a crosshead speed of 5mm/min with a 500 kgf load cell, engaged the centre of the upper surface of the specimen. The specimens

were loaded until fracture occurred. The peak load (fracture load) was recorded in chart recorder.

The Peak load is converted to flexural strength by the formula:

$$S = 3PL/2bd^2$$

Where S= Flexural strength (N/mm<sup>2</sup>)

P = load at fracture

L = distance between jig supports

b = specimen width

d = specimen thickness

The results were tabulated, subjected to statistical analysis and compared.

### Assessment of Antimicrobial property

The artificial saliva was discarded and the specimens were subjected to the antifungal and antibacterial activity assays using Agar disc diffusion method.

### Antimicrobial property against Candida Albicans

For obtaining the inoculum, the reference strains (Candida albicans ATCC 28366) were inoculated in Mueller Hinton Broth. After incubation at 37°C for 24 hours, the turbidity of Candida albicans inoculum was adjusted to tubes 1 McFarland standard. Mueller Hinton Agar plates were swabbed using sterile cotton swab dipped in 1 McFarland matched Candida albicans culture in Mueller Hinton broth. Specimens measuring 6mm in diameter and 1mm in thickness were placed in 6mm diameter wells prepared on surface of Mueller Hinton Agar plates. The plates were kept at room temperature for 120 min for diffusion of the antimicrobial agent and then incubated in aerobiosis at 37° C for 24 hours. The zone of inhibition diameters were measured with zone measuring scale (in mm) and this measurement indicated the microbial susceptibility to the material.

the turbidity of Staphylococcus aureus inoculum was adjusted to tubes 0.5 McFarland standard. Mueller Hinton Agar plates were swabbed using sterile cotton swab dipped in 0.5 McFarland matched Staphylococcus aureus culture in Mueller Hinton broth. Specimens measuring 6mm in diameter and 1mm in thickness were placed in 6mm diameter wells prepared on surface of Mueller Hinton Agar plates. The plates were kept at room temperature for 120 min for diffusion of the antimicrobial agent and then incubated in aerobiosis at 37° C for 24 hours. The zone of inhibition diameters were measured with zone measuring scale (in mm) and this measurement indicated the microbial susceptibility to the material. The results were tabulated, subjected to statistical analysis and compared.

### Statistical Analysis

Following statistical methods were applied in analyzing the results of the variables considered in the study.

- Descriptive statistics
- Independent sample 't' test
- Scheffe's Post Hoc test
- Analysis of variance

Statistical analysis were conducted with SPSS (version 16). Table 1 shows the One- way ANOVA analysis which demonstrates a highly significant difference (p=.000) between the control and the test groups which were fabricated using different percentages of nano clay and silver nano particles. Table 2 shows the One-way ANOVA analysis which demonstrates a highly significant difference (p=.000) between the control and the test groups which were fabricated with 3% nano clay and different percentages of silver nano particles.

## RESULTS

The results of the study showed statistically significant decrease of flexural strength in comparison to the control

Table 1. One way Anova- Group

Oneway Anova flexural strength					
	Sum of Squares	df	Mean Square	F	Significance
Between groups	2301.388	2	1150.694	21434.138	.000
Within groups	1.449	27	.054		
Total	2302.837	29			

Table 2. One way anova- group (antimicrobial property)

Oneway Anova						
		Sum of Squares	df	Mean Square	F	Significance
Candida Albicans	Between Groups	23.700	2	11.850	711.000	.000
	Within Groups	.200	12	.017		
	Total	23.900	14			
Staphylococcus Aureus	Between Groups	52.500	2	26.250	15750.000	.000
	Within Groups	.020	12	.002		
	Total	52.520	14			

### Antimicrobial property against Staphylococcus Aureus

For obtaining the inoculum, the reference strains (Staphylococcus aureus ATCC 25293) were inoculated in Mueller Hinton Broth. After incubation at 37°C for 24 hours,

group on addition of 3% nano clay and 0.5% silver nano particles. It was followed by statistically significant increase of flexural strength on addition of 3% nano clay and 1% silver nano particles, but lesser when compared to the control group.

Statistically significant increase in antimicrobial property was observed with the addition of 0.5% and 1% of silver nano particles along with 3% nano clay to the conventional PMMA denture base resin. The antimicrobial property increases as the concentration of silver nano particles increases within the denture base resin.

## DISCUSSION

The flexural properties of denture base materials are of importance for predicting their clinical success during function. Dentures are commonly subjected to flexures during masticatory function. Repeated flexures can lead to failure in well fitting denture occluding against natural teeth. Flexural strength is important because it reflects the rigidity of the material, which in turn is important for the integrity of the supporting ridges and tissues, along with the fitting accuracy of the denture.<sup>9</sup> Decreased flexural strength of the denture base resin will lead to its fracture when subjected to flexural stress. Nano clay dispersed into the PMMA matrix improves the structure, flexural strength and thermal stability of PMMA,<sup>4</sup> and may decrease the possibility of fracture of the denture base resins. Nano clay being organic in nature may increase the chances of microbial colonization after its incorporation into the denture base resin. To compensate for this, incorporation of an antibiotic agent is necessary.

Silver nano particles have been used for their antimicrobial effect in different biomedical applications,<sup>10</sup> also in modifying commercial acrylic resins but not in the formulation of an experimental acrylic for denture bases. Silver nano particles are also reported to be nontoxic to humans and very effective against bacteria, viruses, and other eukaryotic micro-organisms at very low concentrations and without side effects.<sup>11</sup> These silver nano particles can be used as antimicrobial control systems and are also stable within PMMA matrix of the acrylic denture base resin. Addition of nano clay and silver nano particles may provide us with an improved acrylic denture base resin which possesses better mechanical property and antimicrobial activity. In the present study, 3% nano clay and varied concentrations of silver nano particles were added in to the heat polymerized acrylic resin to evaluate the flexural strength and antimicrobial property of the conventional PMMA denture base resin.

### Evaluation of flexural strength

A statistically significant decrease of flexural strength in comparison to the control group was observed with the addition of 3% nano clay and 0.5% silver nano particles. This was followed by increase in flexural strength with addition of 3% nano clay and 1% silver nano particles, to the acrylic resin, but lesser when compared to the control group, which is consistent with the findings of previous investigations done using AgNps as antimicrobial agents (*A. sodagar et al 2012*). The decrease of flexural strength values in this study followed by increase, in agreement with the results of the study done by *Sodagar et al*, who reported that probably, at low concentration, particle dispersion and chemical interactions between PMMA and AgNps are low which result in flexural strength reduction. We can say that here, at the 0.5%

concentration, AgNps act as impurities which usually decrease mechanical strength in the composites. Along with increase in concentration of the nano particles, the more chemical interaction between the C=O groups and the silver overcome the aforementioned negative effects, leading to the flexural strength improvement.<sup>12</sup> However, it is important to realize that the least mean flexural strength value (84.29 MPa) obtained for the samples in which the percentage of nano clay and silver nano particles was 3% and 0.5 % is more than the minimum flexural strength value of 65 MPa set forth by the ADA specification no.12. Hence this slight reduction may not have any clinical relevance.<sup>13</sup>

### Evaluation of antimicrobial property

It was observed that the control specimens (fabricated without incorporation of nano clay and silver nano particles), did not have antimicrobial activity, which agrees with the results of previous studies that report little or no antimicrobial activity of the tested materials. The addition of 0.5% of silver nano particles with 3% nano clay to heat polymerized acrylic resin was enough to provide antimicrobial activity against the test strains. A significant increase in antimicrobial property was observed with the increase in the concentration of silver nano particles along with nano clay at constant concentration. The higher, the percentage of silver nano particles, the greater the antimicrobial property. The smallest inhibition halos were observed for the heat polymerized acrylic resin specimens with 0.5% of silver nano particles with 3% nano clay against *Candida albicans* and intermediary for *Staphylococcus aureus* isolate, while the largest inhibition halos were observed for heat polymerized acrylic resin specimens with 1% of silver nano particles with 3% nano clay against *Staphylococcus Aureus* isolate followed by *Candida Albicans* isolate for the same. These results may be attributed to silver nano particles present in the acrylic resin. The major advantage of this addition could be for the elderly people with restricted manual dexterity or cognitive disturbances. Also, since the benefits of silver nano particles along with nano clay outweigh the toxicity risks, the use of these nano particles in denture base resin can be recommended. An antimicrobial biocompatible PMMA was developed as a denture base.

### Conclusion

The flexural strength of conventional PMMA denture base resin after incorporation of 3% nano clay and 0.5% silver nano particles showed the least flexural strength. Incorporation of 3% nano clay and 1% silver nano particles showed higher flexural strength but lesser compared to the control group. The antimicrobial property of conventional PMMA denture base resin after incorporation of 3% nano clay and 0.5% silver nano particles showed lower antimicrobial property but higher compared to the control group. Incorporation of 3% nano clay and 1% silver nano particles showed the highest antimicrobial property. A modified acrylic denture base resin was synthesized having sufficient flexural strength which was more than the minimum flexural strength value set forth by the ADA specification no.12. It was significant even with the addition of small percentages of silver nano particles to denture base acrylic resin along with nano clay showed good amount of antimicrobial property. This property is highly beneficial for

patients who do not follow a sufficient denture cleaning protocol. The major advantage of this addition could be for the elderly people with restricted manual dexterity or cognitive disturbances.

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