

Comparative Assessment of Flexural Strength in Heat-cured Polymethyl Methacrylate Resin Reinforced with Silver Nanoparticles, Siwak, and Fluconazole: An In-vitro Study

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ABSTRACT

Introduction: For crafting complete dentures, Polymethylmethacrylate (PMMA) has long been the material of choice. Various reinforcements are being used to enhance the properties of PMMA and minimise denture fracture.

Aim: The present in-vitro study compared the flexural strength of PMMA resin samples reinforced with Silver Nanoparticles (Ag NP), siwak, and fluconazole.

Materials and Methods: The present in-vitro experiment was conducted from July to August 2017 at the Prosthodontics Department of a Dental College with technical support from the Centre for Scientific Research and Development (CSR), affiliated with People's University in Bhopal, Madhya Pradesh, India. Flexural testing was conducted at the Central Institute of Plastic Engineering and Technology (CIPET), Bhopal. A total of 120 acrylic resin samples, each measuring 65 mm in length, 10 mm in width, and 2.5 mm in thickness, were fabricated in accordance with American Dental Association (ADA) Specification No. 12. These specimens were categorised into four distinct groups. The first group consisted of only PMMA resin (Group A), while the remaining three groups comprised PMMA resin enhanced with AgNP (Group B), Siwak (Group

C), and Fluconazole (Group D), respectively, with each group containing 30 samples. Subsequently, these specimens underwent a flexural strength assessment through a 3-point flexural test. The statistical analysis to assess and compare the mean flexural strength of the samples was conducted. The present analysis was performed first by applying an Analysis of Variance (ANOVA) test, followed by pairwise comparisons with Tukey's Post-hoc test. A p-value less than 0.05 was used to determine statistical significance.

Results: The mean flexural strength (\pm Standard Deviation) in N/mm² for PMMA; PMMA+AgNP; PMMA+Siwak and PMMA+Fluconazole was 82.33 \pm 5.18; 80.55 \pm 5.67; 71.50 \pm 10.15; and 74.03 \pm 10.84, respectively. The mean flexural strengths of the PMMA and PMMA+AgNP groups were similar ($p=0.842$). However, the flexural strength of the Siwak+PMMA and Fluconazole+PMMA groups was significantly less than that of the other two groups.

Conclusion: The flexural strength of PMMA is unaffected by the inclusion of AgNP at a concentration of 1%, and its incorporation would allow the benefit from its antibacterial qualities. It is not advised to add fluconazole or siwak because doing so reduces the flexural strength.

Keywords: 3-Point flexural test, Antibacterial properties, Dental materials, Mechanical properties

INTRODUCTION

The world is undergoing a demographic transition in which the elderly population is increasing. This rise in the ageing population poses challenges for dental care, particularly in catering to edentulous patients. Dental implants are often considered the preferred treatment choice for individuals with complete or partial tooth loss. However, in practice, individuals often choose conventional complete dentures. This decision is influenced by various factors, including the patient's financial limitations, physical constraints, and underlying biological conditions [1,2]. Therefore, considering the need for the same, advancing research in dental materials has amplified the scope for prosthetic and restorative applications. Due to its numerous advantages, PMMA has long been the material of choice for crafting complete dentures. These include its cost-effectiveness, lightweight nature, ease of processing, and quick repairability [1,3]. An ideal denture base material should possess enhanced aesthetics while exhibiting superior properties, including a higher modulus of elasticity, strength (flexural and impact), and the capacity to be easily repaired or adjusted for contour changes. Additionally, dimensional stability is a crucial characteristic to be sought in such a material [1,4,5].

Denture fracture is a known problem. Improvement in flexural and impact strength of PMMA to prevent fracture has been reported in the literature. Researchers have endeavoured to enhance the

properties of PMMA by modifying its composition or reinforcing using more robust materials. These approaches involve incorporating copolymers to alter the design and reinforcing dentures with aramid, carbon graphite, polyethylene, and glass fibers. Additionally, there has been exploration into enhancing PMMA by reinforcing it using metallic fillers and nanoparticles, including materials such as silica and titania nanoparticles [1,6-13]. Silver, known for its benign nature towards oral mucosa and potent antimicrobial properties against *Candida albicans*, has been extensively explored as a filler material in dental research. Various studies have investigated the mechanical properties of PMMA when reinforced with AgNP at different concentrations. The outcomes of these studies [14-21] have shown a range of effects on the flexural strength of PMMA, with some indicating a decrease, others an increase, and several showing no significant change. Notably, the specific impact of adding 1% AgNP has not been thoroughly investigated. Alongside silver, Fluconazole, a well-known antifungal agent, and Siwak, recognised for its antimicrobial properties, have also garnered interest [22-25].

However, there remains a gap in understanding how the integration of these substances into denture materials influences their mechanical integrity, particularly in terms of flexural strength. The present study aimed to bridge this gap by assessing whether the antimicrobial benefits of these agents can be incorporated into denture materials

without adversely affecting their essential mechanical properties. The objective of present study was to compare and evaluate the flexural strength of pure heat-cured PMMA resin when reinforced with AgNP, Siwak, and Fluconazole.

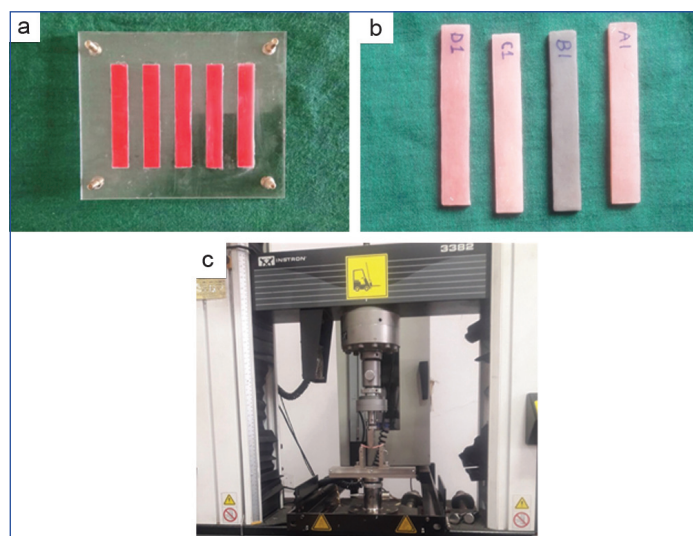
MATERIALS AND METHODS

This in-vitro experiment was conducted from July to August 2017 at the Prosthodontics Department of a Dental College with technical support from the Centre for Scientific Research and Development (CSR), affiliated with People's University in Bhopal, Madhya Pradesh, India. Flexural testing was conducted at the Central Institute of Plastic Engineering and Technology (CIPET), Bhopal. The study protocol was approved by the Research Advisory Committee of PDA, Bhopal (Approval number: ICC/Ref. No./2015/3 Date 17 Dec 2015).

Sample size calculation: G-Power software was used to calculate the sample size [26]. The required sample size to test whether mean flexural strength is different among four groups, assuming a medium effect size, type-I error of 5%, and power of 80%, was 30 samples in each group, totaling 120 samples. Therefore, it was decided to fabricate 120 specimens and divide them into four groups of 30 specimens each.

Study Procedure

Specimen Fabrication: In accordance with ADA Specification No. 12, 120 acrylic resin samples were fabricated, each measuring 65 mm in length, 10 mm in width, and 2.5 mm in thickness [27], following the recommended procedure [Table/Fig-1a,b]. [Table/Fig-2,3] show the details of the materials and equipment used for this study.



[Table/Fig-1]: Specimen fabrication and testing. a) Wax specimen in fabrication in standardised mould; b) Fabricated specimens of four groups; c) Specimens under three point bending test in Universal testing machine.

Material	Trade name/manufacturer	Material type
Denture base resin (veined) (Figure-X)	Trevalon	Polymer and monomer
Silver Nanoparticles (Ag, Purity 99.9%, 30-50 nm, metal basis)	Nano Labs, H 21, Gopalpur, Jadugoda, (E) Singhbhum, Jamshedpur, Jharkhand 832102 India	Powder
Siwak (Salvadora persica)	Haleem Sewak Miswak Tooth Stick	Sticks from which powder was made
Fluconazole	Fluka 150 tablet	Powder obtained from tablet

[Table/Fig-2]: Details of material used in study.

To facilitate the easy removal of the wax specimens, petroleum jelly was applied as a lubricant to the mould. The process involved joining the lower and middle plates, and then pouring molten wax

Materials	Silver Nanoparticles water dispersion (AgNps aqueous dispersion)
Purity	99.90%
CAS# (Chemical Abstract Service Database Number)	7440-22-4
Molecular formula	Ag
Appearance	Tawny
Odour	Odourless
Average particle size	10-20 nm
pH	6-7
Melting point	960.8°
Density	10.491 g/cm ³

[Table/Fig-3]: Technical specifications of Silver nanoparticles (AgNP).

into the mould. Subsequently, the upper plate was positioned, and the entire assembly was subjected to pressure using a bench press to eliminate any excess wax. After lifting the upper plate, a sharp BP knife was utilised to remove excess wax. After detaching it from the platform, gentle pressure on the open top was applied to extract the solidified wax from the mould. All resulting specimens exhibited uniformity and consistency in all dimensions. Any specimens that appeared damaged, broken, or distorted were excluded from the analysis. The prepared wax patterns were thoroughly washed in a soap solution and then made ready for flasking.

Processing of Acrylic Resin Specimens [28,29]:

Investing of the wax patterns: The dental stone was used to invest the wax patterns in standard brass flasks. Each flask accommodated five patterns. A separating medium was used after the dental stone had been set, and a second pour was performed. After that, a clamp was used to close and firmly tighten the flask. The setting time for each flask pour was about an hour.

Dewaxing of the patterns: Following the placement of the flask assembly in the dewaxing unit for 7-8 minutes, the segments of the flask were segregated and washed completely with hot water to eliminate any remaining wax. Subsequently, the moulds were cleaned by washing them with soapy water and then left to air dry. While the moulds were still warm, a thin, even layer of a separating medium (a substitute for tin foil) was meticulously applied to the plaster surfaces on flask parts to ensure uniformity.

Mixing and Packing of Control Group (Group A) Specimens:

After cooling, the denture base resin (specifically, Trevalon HI, Dentsply India Private Limited, Batch No: TH70102), available in both powder and liquid (monomer) forms, was meticulously mixed in a porcelain jar, following the manufacturer's guidelines. The mixture was carefully brought to the dough stage, kneaded, and then inserted into the mould. Subsequently, a trial closure was performed using a hydro press applying a force of 40,000 N [8]. The flask was secured, and a controlled, low-pressure environment was maintained for 30 minutes. This allowed for effective penetration of the monomer into the polymer, ensuring consistent material flow and removing excess material. A total of 30 specimens were prepared using this method.

Mixing and Packing of Experimental Group (Group-B):

Silver nanoparticles+Polymethacrylate Denture Base Resin [14] Nano Labs Pvt., Ltd., supplied commercially available AgNP in powder form, and their properties are detailed in [Table/Fig-2]. To ensure purity, a laboratory certification was obtained. For the experiment, 2.1 grams of AgNP, constituting 1% of the weight, were added to the liquid monomer. Using a magnetic stirrer, the liquid monomer was continuously stirred for approximately 15-20 minutes. This process adhered to the manufacturer's recommended powder-liquid ratio of 21 grams to 10 mL. Once the mixture had reached the dough stage, it was carefully kneaded and then placed into the mould.

To complete the process, a trial closure was performed using a hydro press with a pressure of 40,000 N [8]. Subsequently, the flask was securely clamped, and controlled low pressure was maintained for 30 minutes. This step was essential to ensure effective penetration of the monomer into the polymer, guaranteeing uniform material distribution and removing any excess material. In total, 30 specimens were crafted using this methodology.

Group-C: Siwak (*Salvadora persica*)+Polymethacrylate Denture Base Resin [24] Siwak sticks were ordered from an e-commerce platform and kept in a desiccator for three weeks to dry. Then, their outer coverings were peeled, and the remaining parts were crushed and put in an electric grinder to prepare a powder form, which was then mixed with monomer by 1% weight, i.e., 2.1 gm of Siwak powder. The mixture was then stirred with a magnetic stirrer. Further procedures were carried out similarly to those in Groups A and B.

Group-D: Fluconazole+Polymethacrylate Denture Base Resin [22] Fluconazole tablets (Zocon 150 mg IP, Manufacturer- FDC) were crushed, and a powder form was prepared. 1% of the weight, i.e., 2.1 gm, was mixed with the monomer liquid and stirred with a magnetic stirrer. The procedure followed that of Groups A, B, and C. The final closure was executed at a pressure of 2750 psi. Subsequently, the flask was subjected to bench curing for one hour. Following this, the flasks were immersed in acrylic, and the curing process took place at 74°C for two hours, followed by an additional hour at 100°C. The flasks were removed and allowed to bench cool overnight. Specimens were retrieved after complete cooling and finished and polished with silicon carbide paper. Mechanical testing was conducted using a Universal Testing machine, which was accessible in the laboratory of CIPET Bhopal.

Flexural Strength Testing: Total 30 specimens from each group underwent a thorough assessment of their flexural strength via a 3-point flexural test. These samples were meticulously positioned in a calibrated Instron Universal Testing Machine, supplied by Instron Corp., Canton, MA. This testing apparatus included a loading wedge and adjustable supporting wedges set 48 mm apart.

During the test, each specimen was precisely centered on the equipment, ensuring that the loading wedge engaged the center of the upper surface of the sample (as depicted in [Table/Fig-1c]). The loading wedge advanced at a controlled cross-head speed of 2 mm per minute. The specimens were subjected to loading until they fractured, at which point the peak load (fracture load) was accurately recorded using a chart recorder.

The flexural strength (S) of each specimen was calculated using the formula: $S = 3PL/2bd^2$, where S represents the flexural strength in N/mm², P is the load at fracture, L is the distance between the jig supports, b represents the specimen width, and d signifies the specimen thickness [30].

STATISTICAL ANALYSIS

All data readings were entered into an Excel spreadsheet and subsequently analysed using R version 4.2.2. For exploratory data analysis, descriptive statistics were computed, encompassing measures of central tendency such as the mean and median, and measures of spread such as standard deviation or interquartile range.

Then, the mean flexural strength across the four groups was compared by applying the Analysis of Variance (ANOVA) test, which informed if there was any difference in the distribution of flexural strength. Pairwise comparisons were done using Tukey's Post-hoc test to identify which pairs differed significantly. To ascertain statistical significance in all analyses, a significance level of $p < 0.05$ was adopted.

RESULTS

The flexural strength values and corresponding descriptive statistics for each experimental group are presented in [Table/Fig-4a,4b]. In

The distribution of flexural strength was significantly different across the four groups ($p < 0.001$). Furthermore, Tukey's test was applied for pairwise comparisons. [Table/Fig-5] depicts flexural strength across the four groups through a Box-Violin-Jitter plot. The average flexural strength of the PMMA and the PMMA+AgNP groups was comparable ($p = 0.842$, Not Significant). However, the average flexural strength of

Descriptive statistics	PMMA	PMMA+ AgNP	PMMA+ Siwak	PMMA + Fluconazole
Mean	82.3	80.6	71.5	74.0
Standard Deviation	5.2	5.7	10.2	10.8
Median	81.9	80.4	71.8	73.9
Percentile 25	79.0	76.0	66.3	67.3
Percentile 75	86.5	85.7	75.7	83.3
Minimum	72.5	69.5	46.1	52.7
Maximum	91.8	89.9	96.2	91.2

[Table/Fig-4a]: Descriptive statistics for flexural strength among different study groups.

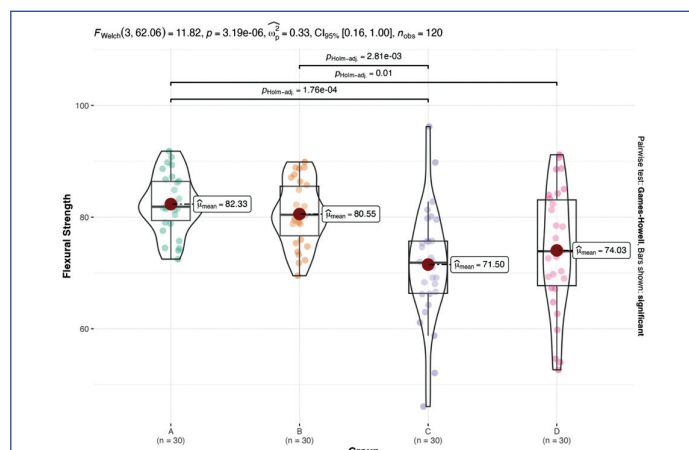
One-way ANOVA Test statistics	Sum of squares	df	Mean square	F	p-value.
Between groups	2400.792	3	800.264	11.471	<0.001
Within groups	8092.537	116	69.763		
Total	10493.329	119			

[Table/Fig-4b]: One-way ANOVA test statistics.

(I) Study group	(J) Comparison group	Mean difference (I-J)	p-value	95% Confidence Interval	
				Lower bound	Upper bound
PMMA	AgNP	1.78	0.842	-3.84	7.40
PMMA	Siwak	10.83	<0.001	5.21	16.45
PMMA	Fluconazole	8.29	0.001	2.68	13.92
AgNP	Siwak	9.05	<0.001	3.43	14.67
AgNP	Fluconazole	6.51	0.016	0.89	12.14
Siwak	Fluconazole	-2.53	0.644	-8.16	3.09

[Table/Fig-4c]: Tukey's Post-hoc test for comparison of flexural strength (in N/mm²) among each groups.

Group A, which consisted of specimens without reinforcement, the mean flexural strength was determined to be 82.33 N/mm², with a standard deviation of 5.18 N/mm². For the PMMA specimens with reinforcement, the mean flexural strength (\pm Standard Deviation) of Ag NP, Siwak, and Fluconazole was 80.55 \pm 5.67 N/mm², 71.50 \pm 10.15 N/mm², and 74.03 \pm 10.84 N/mm², respectively. The strength of the PMMA+Siwak and PMMA+Fluconazole groups was similar ($p = 0.644$). This means that Siwak and Fluconazole had a similar impact in terms of material strength [Table/Fig-4c].



[Table/Fig-5]: Box-Violin-Jitter plot showing distribution of flexural strength across four groups.

the PMMA+Siwak and PMMA+Fluconazole group was lower than only the PMMA group ($p < 0.001$, Highly Significant). This suggests that the addition of Siwak or Fluconazole had a noticeable negative impact on the material's strength. Also, the mean flexural strength of the PMMA+Siwak and PMMA+Fluconazole group was lower than the PMMA+AgNP group ($p < 0.001$ and $p = 0.016$, respectively; Highly Significant). This shows that AgNP were more effective at maintaining strength compared to Siwak and Fluconazole.

DISCUSSION

The present in-vitro study reports that flexural strength remains similar even after reinforcement with AgNPs. However, flexural strength declines with the reinforcement of Siwak and Fluconazole. Various reinforcements are essential to improve the mechanical, physical, and other essential characteristics of PMMA dentures while promoting biocompatibility and reducing failure rates. Notably, a significant portion of clinical failures associated with PMMA dentures can be attributed to fractures, which may result from either fatigue or impact forces [22,23]. PMMA lacks some mechanical properties and is thus prone to fractures, and its reinforcement with suitable polymers would reduce the likelihood of fractures [6,31,32].

Numerous studies have tested mechanical properties after PMMA reinforcement by adding AgNP in different concentrations [14-21]. Reduced flexural strength of PMMA resin was reported with AgNP concentrations of 0.05% [17], 0.2% [18], and 2% [18], and an increase was reported at 0.5% [16]. In contrast, no change was reported at AgNP concentrations of 0.8% and 1.6% [14]. A meta-analysis investigating the impact of AgNP addition on flexural strength has concluded that the flexural strength tends to either decrease or show no significant improvement when AgNPs are incorporated [33]. In present study, no decrease in flexural strength after adding 1% AgNP was observed. This variation of results in a change in flexural strength was attributed to different types of resin, quantity, concentration, and brands of resins, and polar interactions formed between C=O [16,17]. Since flexural strength is not negatively affected with 1% AgNP, its use in reinforcement would help gain benefits of the antibacterial properties of silver. Modern dentistry has a prominent trend in using medication extracted from natural plants as biocompatible agents [34]. As a growing interest in medicinal plants for therapeutic application, Siwak was used as a filler in denture base resin material.

Abdul-Rahman Khalaf H conducted a study to assess the impact of incorporating Siwak powder, with an average particle size of 75 micrometers, into PMMA. This investigation involved the use of three distinct weight-based concentrations of Siwak powder to determine its effects on various mechanical properties of PMMA. Their investigation demonstrated that incorporating Siwak powder at 3% and 5% by weight ratios into the heat-polymerising acrylic resin did not adversely impact its physical and mechanical properties. However, when 7% Siwak powder by weight was introduced into the heat-polymerising acrylic resin, it had an adverse effect on most of the tested mechanical properties, except for the transverse test [24]. Recently, Haitham R and Mohammed R reported the effect on the flexural strength of PMMA reinforced with 1%, 2%, 3%, and 4% Siwak particles [25]. They reported similar flexural strength with 1% Siwak particles but a marked increase with 4% concentration; however, their sample size for each group was smaller ($n = 4$ per group).

In the current study, adding 1% Siwak to the PMMA significantly affected its flexural strength. Siwak contains silica as its main abrasive ingredient, along with sodium bicarbonate [35]. The presence of Siwak particles may influence the bonding of polymer chains due to potential variations in surface tension caused by their entry into the polymer chains. Although the results of this study differ from the

previous one, it is important to note that Siwak exhibits antibacterial activity against numerous oral pathogenic bacteria [36].

Antimicrobial agents in dental materials are also a trend in dentistry, along with the incorporation of bioactive compounds [37]. In the present study, Fluconazole was the drug of choice as a filler material for its antifungal activity against oral microorganisms, including candida. The treatment of denture stomatitis caused by fungal infection is always a challenge for prosthodontists. Soft liners and acrylic resin incorporated with antifungal agents were explored, and their benefits, such as the continued presence of drugs at the site of action and feasibility for use, are suggested [38]. It was found that it has better effects, such as the continued presence of drugs at the site of action.

Al-Haddad A et al., investigated the influence on the fracture toughness of PMMA after adding bioactive compounds such as Fluconazole and Chlorhexidine. They discovered that adding 4.5% by mass of Fluconazole did not change the fracture toughness of the PMMA [23]. On the other hand, in another study by Yadav NS et al., it was found that adding 10% by mass of Fluconazole resulted in lower values of the denture base resin's flexural strength [22].

In present study, it has been found that the incorporation of 1% Fluconazole led to a decrease in the flexural strength compared to the control group specimens. These differences were statistically significant and had an impact on the physical properties of the denture.

The clinical implications of present study indicate that the addition of AgNP has no detrimental impact on the flexural strength of denture base resins, irrespective of the percentage of nanosilver added. However, it has been observed that the addition of Siwak and Fluconazole negatively affects the acrylic resin, potentially compromising the strength and durability of complete dentures.

Limitation(s)

The in-vitro nature of the study, although precise for controlled experimentation, may not fully emulate the complex oral environment, where variables such as saliva, temperature, and mechanical forces play a significant role. The study's focus on immediate flexural strength does not encompass the long-term durability and wear resistance of the PMMA composites, which are essential for their practical application in dentistry. Furthermore, the investigation is restricted to specific additives, limiting the exploration of a broader range of materials that could potentially enhance PMMA's properties. Finally, the absence of clinical correlation limits the direct applicability of the findings to patient care, underscoring the need for future research that includes clinical evaluations to substantiate the laboratory results.

CONCLUSION(S)

The flexural strength of PMMA is unaffected by the inclusion of AgNP at a concentration of 1%, and its incorporation would allow for the benefit from its antibacterial qualities. It is not advised to add fluconazole or Siwak because doing so reduces the flexural strength.

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