

## Original Article

# Effect of Green Gold Nanoparticles Synthesized with Plant on the Flexural Strength of Heat-polymerized Acrylic Resin

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## INTRODUCTION

Polymethyl methacrylate (PMMA) is the material of choice for almost all removable dental prostheses. However, PMMA has some limitations, such as poor strength and low fracture resistance and microbial adhesion. To overcome these limitations, micron-sized ceramic or metal particles have been incorporated into PMMA, and when this has been insufficient, nanoparticles have been added.<sup>[1-3]</sup> Nanoparticles have a high surface area in relation to their volume, which gives them a high degree of contact with bacteria and fungi. By binding to microbial DNA, nanoparticles are able to inactivate bacteria and inhibit their replication.<sup>[4,5]</sup>

“Polymer nanocomposite” is the term used to describe a polymer that has nanoparticles dispersed within it.<sup>[6]</sup> Different polymer nanocomposites have been developed using different nanoparticles and base polymers.<sup>[7-9]</sup> Metal nanoparticles, including gold, silver, and copper nanoparticles, have demonstrated strong biocidal impact on various bacterial species such as *Escherichia coli*.<sup>[4,10-13]</sup> Although the antimicrobial effect

## ABSTRACT

**Purpose:** The aim of this study was to investigate the effect of gold nanoparticle on the flexural strength of polymethyl methacrylate (PMMA). **Materials and Methods:** PMMA specimens (65 mm × 10 mm × 3.3 mm) containing different sizes (45 nm, 55 nm, and 65 nm) and concentrations (0.05% and 0.2%) of gold nanoparticles were prepared, along with a control group containing no added nanoparticles. Flexural strength of all specimens was measured, and one-way ANOVA and Tukey–Kramer *post hoc* multiple comparisons tests were performed to identify statistical differences between groups. **Results:** The addition of gold nanoparticles increased the flexural strength of acrylic resin. Significantly greater increases were obtained with lower concentrations (0.05%) when compared to higher concentrations (0.20%). **Conclusion:** Differences in concentrations of gold nanoparticles added to PMMA have significantly different effects on PMMA flexural strength, whereas differences in sizes of gold nanoparticles added to PMMA do not significantly affect its flexural strength. Accordingly, adding gold nanoparticles to PMMA may enhance the mechanical properties of denture bases used in clinical practice.

**KEYWORDS:** Flexural strength, gold, nanoparticles, polymethyl methacrylate

of gold is weaker than that of silver,<sup>[14]</sup> gold is the metal most commonly used in both glass and polymer nanocomposites because it is not only biocompatible but also easily synthesized and exceptionally stable.<sup>[15-18]</sup>

In general, nanoparticles are prepared by conventional methods (chemical and physical methods) which involve the use of toxic chemicals that are responsible for various biological risks.<sup>[19]</sup> Synthesis of metal nanoparticles using plant extracts (green synthesis) has advantages over conventional methods involving chemical agents and an environment-friendly method without the use of harsh and toxic chemicals.<sup>[20-23]</sup>

PMMA modulus and strength as well as ductility have been shown to improve with the addition of nanostructuring materials.<sup>[1,2]</sup> However, it is possible

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that the addition of AuNPs as an antimicrobial agent may have a negative effect on the mechanical properties of PMMA.<sup>[24]</sup> To date, no published study has reported on how the addition of AuNPs affects the mechanical properties of heat-polymerized acrylic resin. Therefore, this study evaluated and compared the effects of various sizes and concentrations of AuNPs on the flexural strength of heat-polymerized acrylic resin. It was hypothesized that (1) the addition of AuNPs of different concentrations would have different effects on the flexural strength of PMMA and (2) the addition of AuNPs of different sizes would have different effects on the flexural strength of PMMA.

## MATERIALS AND METHODS

### *Diospyros kaki* leaves extract broth preparation

Persimmon *Diospyros kaki* leaves (Hacettepe University, Turkey) were washed before drying at room temperature for 3 days. Dried leaves were cut into small pieces. Five grams of cut leaves was placed into the 300 ml Erlenmeyer flask with 100 ml of distilled water, boiling for 10 min to obtain yellowish brown solution before decanting. The extract broth was stored at 4°C in refrigerator ambient. *D. kaki* extract broth is better to use weekly.

### Biological synthesis of gold nanoparticles By Persimmon *Diospyros kaki* Leaf Broth

Typically, 0.1 mM HAuCl<sub>4</sub> solution was prepared to synthesize gold nanoparticles. Five milliliters of extracted broth was added to 95 ml of 1 mM HAuCl<sub>4</sub> solution which was heated to solution boiling point reducing gold ions (Au<sup>3+</sup>) to Au nanoparticles in the hydrogen tetrachloroaurate solution. Water reflux was installed on the head of reactor. According to other studies, leaf broth concentration, temperatures, and reaction time are dominant parameters which affect the synthesized gold nanoparticle morphology as well as its mean size. Furthermore, reaction temperature affects reduction conversion rate and reaction duration. In high temperatures, 90% conversion rate of reaction is available. Observations were proved that in 95°C of reaction temperature, the reaction time was very faster (even <60 min related to nanoparticle size) than reactions which were carried out at room temperature.<sup>[25]</sup> A Zetasizer instrument (Malvern 3000 Has, USA) was used to determine the particle size.

### Preparation of specimens

Gold nanoparticles of two different concentrations and three different sizes were added to heat-polymerized acrylic resin, and a total of 49 specimens (65 mm × 10 mm × 3.3 mm) were

prepared according to American Dental Association (ADA) specifications (No. 12)<sup>[26]</sup> for testing flexural strength. Specimens were grouped as follows (*n* = 7 per group):

- Group Aa: 45 nm AuNPs at a concentration of 0.05%
- Group Ab: 45 nm AuNPs at a concentration of 0.2%
- Group Ba: 55 nm AuNPs at a concentration of 0.05%
- Group Bb: 55 nm AuNPs at a concentration of 0.2%
- Group Ca: 65 nm AuNPs at a concentration of 0.05%
- Group Cb: 65 nm AuNPs at a concentration of 0.2%
- Group D: No added AuNPs (control group).

AuNPs were mixed with acrylic monomer liquid (3:1 v/v) at room temperature. Before polymerization, specimens were sonicated (Confident Dental Equipments Ltd., Bengaluru, Karnataka, India) for 1 h to ensure proper dispersion of NP. All specimens were stored in distilled water at 37°C for 50 ± 2 h before testing.

### Flexural strength testing

Flexural strength was then measured using a universal testing machine (Zwick/Roell-Z005 Zwick Roell Group, Herefordshire, UK) with three-point loading and application of a constant load at a crosshead speed of 5 ± 1 mm/min until fracture. Flexural strength was determined using the following formula:<sup>[5]</sup>

$$FS = 3Fl/2bh^2$$

where *F* is the maximum load applied (N), *l* is the distance between supports (span length = 50 mm), *b* is the width of the test specimen (10 mm), and *h* is the thickness of the specimen (3.3 mm).

Fractured surfaces were then examined under a scanning electron microscope (SEM) (Hitachi S-4100 FE-SEM/EDS, Tokyo, Japan). Scanning electron microscopy was used to study the distribution of nanoparticles [Figures 1-4].

### Fourier transform infrared measurements

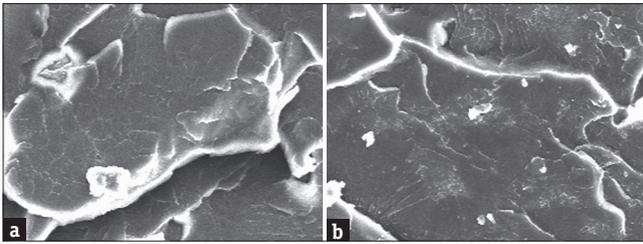
Fourier transform infrared (F-TIR) measurements were used to examine the interaction between polymer and nanoparticles [Figure 5].

### Statistical analysis

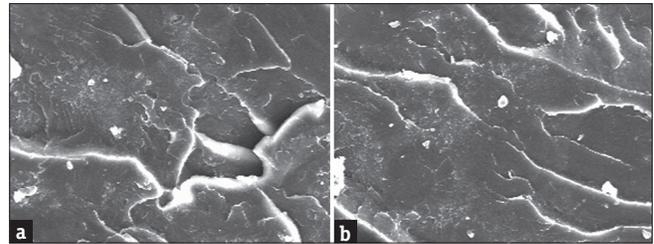
Statistical analysis was performed using one-way ANOVA. Tukey–Kramer *post hoc* multiple comparison tests were used to compare data between groups, with *P* = 0.05 being considered statistically significant [Figure 6].

## RESULTS

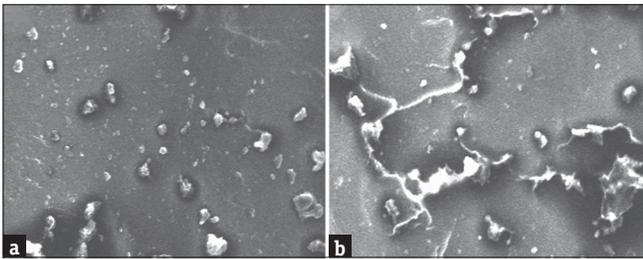
Flexural strengths of the tested materials are



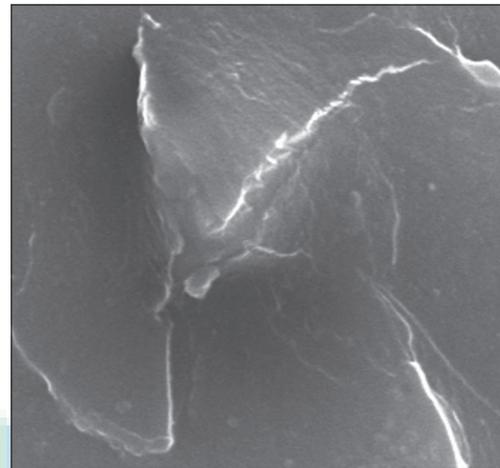
**Figure 1:** (a) Scanning electron microscope image of Group Aa (45 nm/0.05% AuNP); (b) scanning electron microscope image of Group Ab (40 nm/0.2% AuNP), Mag. 500 X



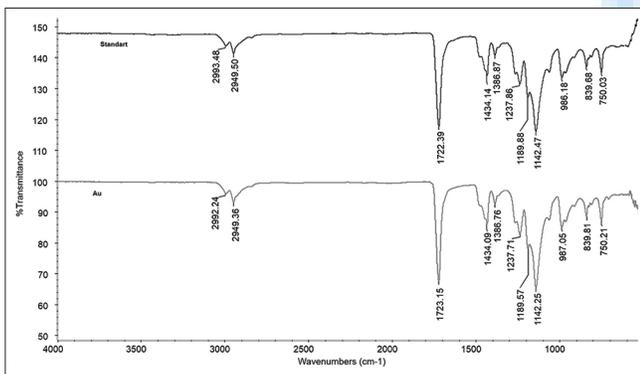
**Figure 2:** (a) Scanning electron microscope image of Group Ba (55 nm/0.05% AuNP); (b) scanning electron microscope image of Group Bb (50 nm/0.2% AuNP)



**Figure 3:** (a) Scanning electron microscope image of Group Ca (65 nm/0.05% AuNP); (b) scanning electron microscope image of Group Cb (60 nm/0.2% AuNP)



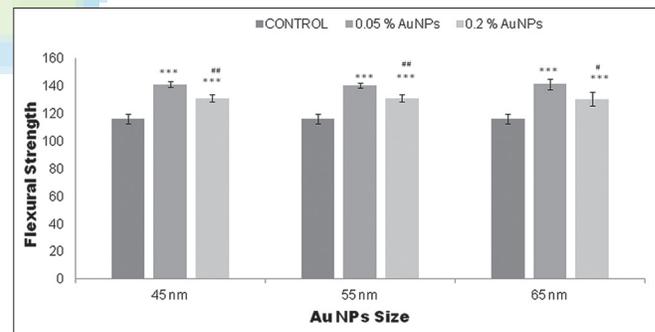
**Figure 4:** Scanning electron microscope image of control group



**Figure 5:** Fourier-transform infrared spectra of polymethyl methacrylate and polymethyl methacrylate/AuNPs. (a) standard, (b) polymethyl methacrylate/AuNPs

shown in Figure 6. All groups containing AuNPs had significantly higher flexural strength when compared to the control group ( $P < 0.001$ ). The groups containing 0.05% AuNPs had significantly higher flexural strength than the groups containing 0.2% AuNPs and the same particle size ( $P < 0.001$ ). However, no significant differences in PMMA flexural strength were observed in the groups containing different sizes of AuNP particles at the same concentrations.

F-TIR measurements indicated no differences between the control group and experimental groups [Figure 5].



**Figure 6:** Statistical comparison of different groups. \* Differences between the control group and other groups. # Differences between polymethyl methacrylate/0.05% Au and polymethyl methacrylate/0.2% Au (\*  $P < 0.05$ , \*  $P < 0.01$ , \*\*\*  $P < 0.001$ , #  $P < 0.05$ , ##  $P < 0.01$ )

## DISCUSSION

This study found that the addition of AuNPs improved the flexural strength of PMMA. However, while different concentrations of AuNPs had significantly different effects on the mechanical properties of PMMA, differences in AuNPs size did not. Thus, the first study hypothesis was accepted, but the second study hypothesis was rejected.

Because denture bases are in direct contact with the oral mucosa, biocompatible materials are required to prevent hypersensitivity and to avoid the release of toxic matter in clinical situations.<sup>[27]</sup> Although silver nanoparticles have demonstrated a broad range of antimicrobial activities, recent studies have reported silver nanoparticles to be cytotoxic, genotoxic, and antiproliferative.<sup>[28]</sup> A study by Pan *et al.*<sup>[29]</sup> also showed gold nanoparticles between 1 and 2 nm in size to have very toxic effects, whereas a number of studies have reported AuNPs ranging between 14 and 100 nm in size to have no cytotoxic effects in mammals.<sup>[30-32]</sup> Therefore, this study was conducted using gold nanoparticles of 45, 55, and 65 nm.

Conventional approaches to nanoparticle synthesis rely on toxic chemicals that result in toxic side effects upon administration. In contrast, “green synthesis” is able to generate nontoxic nanoparticles safely, effectively, and less expensively than conventional methods.<sup>[33]</sup> Therefore, this study relied on “green synthesis” for the production of gold nanoparticles.

The flexural strength of acrylic denture bases is an extremely important issue that has attracted much attention.<sup>[34]</sup> ISO 20795-1 (2008) International Standards have established 50 MPa as the minimum flexural strength required of all acrylic resins used for denture bases. The addition of materials may affect the mechanical properties of acrylic materials, causing the flexural strength to decrease below-standard recommended levels.<sup>[34]</sup>

When nanoparticles are mixed with monomers or polymers, the nanoparticles may aggregate into large clusters, negatively affecting the characteristics of the nanocomposite.<sup>[35-37]</sup> It has been suggested that this may be due to concentrations of stress at the sites of agglomeration.<sup>[37]</sup> When nanoparticles are improperly dispersed within the acrylic resin matrix, monomer reaction decreases, and the amount of unreacted monomers increases.<sup>[36]</sup> It is also likely that stress concentrations caused by filler particles change the resin’s modulus of elasticity and mode of crack propagation.<sup>[37]</sup> By uniformly dispersing the reinforcing agent within the resin, the development of areas of stress concentration may be prevented and the mechanical properties of the resin improved.<sup>[38]</sup> In the present study, after adding AuNPs to the monomer, the mixtures were sonicated for 1 h to ensure proper dispersion of the nanoparticles and prevent agglomeration.

Studies examining how the mechanical properties of acrylic resin are affected by the addition of different nanoparticles have reported conflicting results.<sup>[34,39-42]</sup> However, none of the studies in the literature have examined the effect of gold nanoparticles on the

mechanical properties of PMMA; therefore, it was not possible to compare the results of the present study and those of previous similar studies.

Some studies have reported that the effect of the incorporation of nanoparticles such as Ag, TiO<sub>2</sub>, and SiO<sub>2</sub> on the mechanical properties of acrylic resins is directly correlated with the concentration of nanoparticles, with nanocomposite strength decreasing as nanoparticle concentrations increase.<sup>[39,40]</sup> Similarly, the present study found that PMMA flexural strength was lower with the addition of a higher concentration of AuNPs (0.2%) when compared to a lower concentration of AuNPs (0.05%). In contrast, differences in nanoparticle size were not found to result in significant differences in PMMA flexural strength. Accordingly, it may be concluded that adding AuNPs to PMMA in suitable concentrations may enhance the mechanical properties of denture bases in clinical practice.

The current study is limited by its investigation of only one formulation of acrylic resin and by the fact that as an *in vitro* study, it does not accurately simulate intraoral conditions. Given that the properties of acrylic resins may be affected by factors such as changes in pH and temperature<sup>[43]</sup> that occur in the intraoral environment, predicting the clinical behavior of the material based on its *in vitro* behavior can be difficult. Therefore, in addition to further *in vitro* studies, clinical studies are also required.

## CONCLUSION

Within the limitations of this study, the following conclusions can be drawn:

1. PMMA flexural strength increases with the addition of AuNPs
2. The increase in PMMA flexural strength is greater with the addition of 0.05% AuNPs when compared to 0.2% AuNPs
3. Gold nanoparticle size does not affect PMMA flexural strength.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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