Original Article

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

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Date of Acceptance:
22-May-2018

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*

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.[1-3] 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.[4,5]

"Polymer nanocomposite" is the term used to describe a polymer that has nanoparticles dispersed within it.[6] Different polymer nanocomposites have been developed using different nanoparticles and base polymers.[7-9] Metal nanoparticles, including gold, silver, and copper nanoparticles, have demonstrated strong biocidal impact on various bacterial species such as *Escherichia coli*.[4,10-13] Although the antimicrobial effect of gold is weaker than that of silver,[14] 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.[15-18]

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.[19] 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.[20-23]

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

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_388_17

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How to cite this article: Oyar P, Sana FA, Nasseri B, Durkan R. Effect of green gold nanoparticles synthesized with plant on the flexural strength of heat-polymerized acrylic resin. Niger J Clin Pract 2018;21:1291-5.

that the addition of AuNPs as an antimicrobial agent may have a negative effect on the mechanical properties of PMMA.[24] 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 HAuCl4 solution was prepared to synthesize gold nanoparticles. Five milliliters of extracted broth was added to 95 ml of 1 mM $HAuCl_4$ solution which was heated to solution boiling point reducing gold ions (Au^3+) 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.[25] 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)[26] 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:[5]

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

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

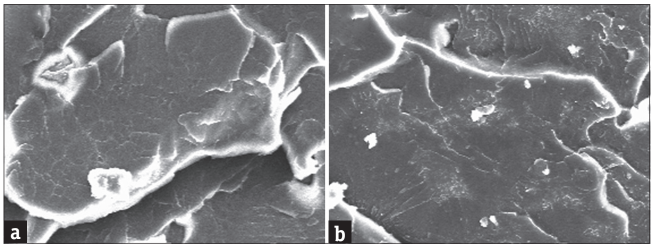

Figure 1: (a) Scanning electron microscope image of Group Aa (45 nm/0.05% AuNP); (b) scanning electron microscope image of Group Ab (40 nm/0.2% AuNP), Mag. 500 X

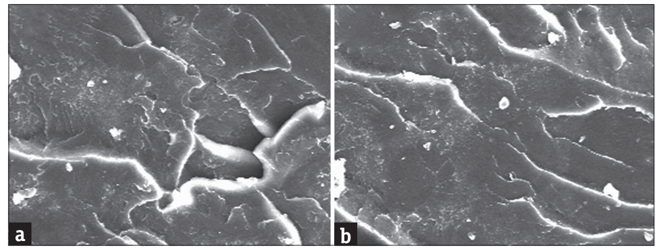

Figure 2: (a) Scanning electron microscope image of Group Ba (55 nm/0.05% AuNP); (b) scanning electron microscope image of Group Bb (50 nm/0.2% AuNP)

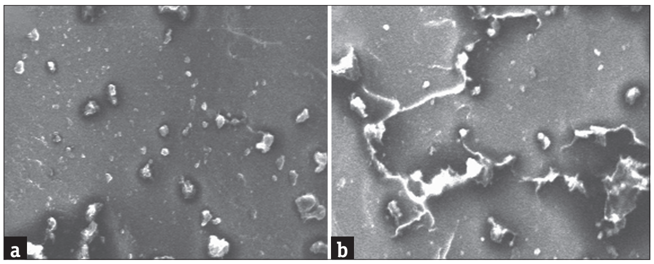

Figure 3: (a) Scanning electron microscope image of Group Ca (65 nm/0.05% AuN); (b) scanning electron microscope image of Group Cb (60 nm/0.2% AuNP)

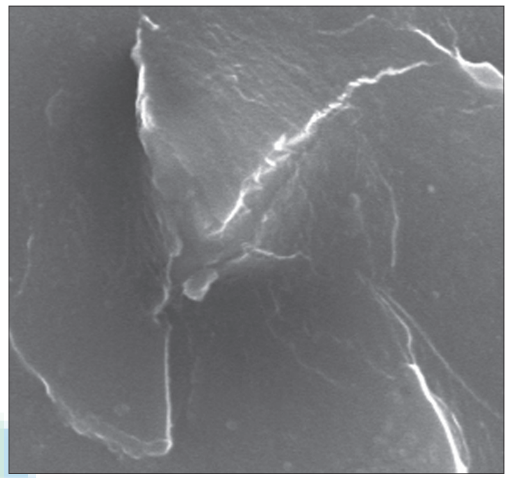

Figure 4: Scanning electron microscope image of control group

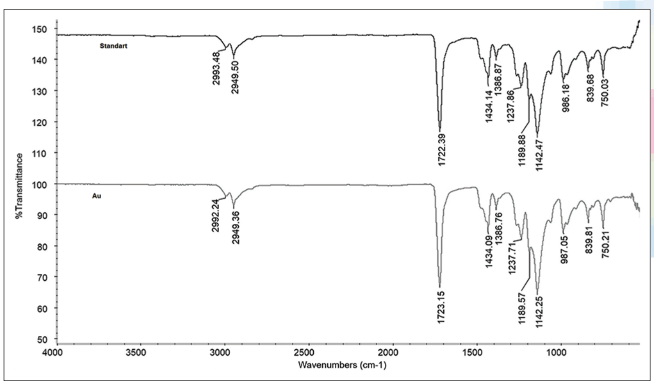

Figure 5: Fourier-transform infrared spectra of polymethyl methacrylate and polymethyl methacrylate/AuNPs. (a) standard, (b) polymethyl methacrylate/AuNPs

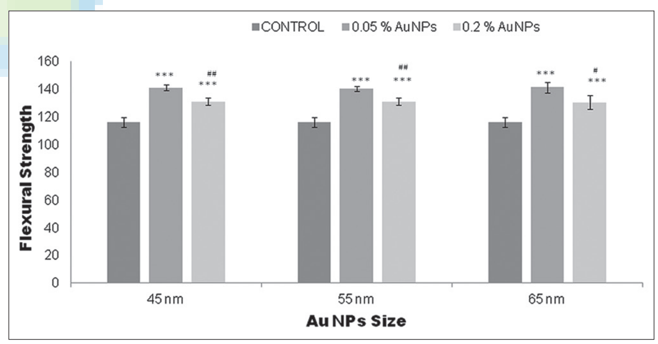

Figure 6: Statistical comparison of different groups. * Differences between the control group and other groups. # Differences between polymethyl methacrylate/0.05% Au and polymethyl methacrylate/0.2% Au (* $P < 0.05$, * * $P < 0.01$, *** $P < 0.001$, # $P < 0.05$, ## $P < 0.01$)

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].

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.[27] Although silver nanoparticles have demonstrated a broad range of antimicrobial activities, recent studies have reported silver nanoparticles to be cytotoxic, genotoxic, and antiproliferative.[28] A study by Pan *et al*.[29] 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.[30-32] 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.[33] 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.[34] 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.[34]

When nanoparticles are mixed with monomers or polymers, the nanoparticles may aggregate into large clusters, negatively affecting the characteristics of the nanocomposite.[35-37] It has been suggested that this may be due to concentrations of stress at the sites of agglomeration.[37] When nanoparticles are improperly dispersed within the acrylic resin matrix, monomer reaction decreases, and the amount of unreacted monomers increases.[36] It is also likely that stress concentrations caused by filler particles change the resin's modulus of elasticity and mode of crack propagation.[37] 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.[38] 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.[34,39-42] 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_2, and SiO_2 on the mechanical properties of acrylic resins is directly correlated with the concentration of nanoparticles, with nanocomposite strength decreasing as nanoparticle concentrations increase.[39,40] 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[43] 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.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Wang W, Liao S, Zhu Y, Liu M, Zhao Q, Fu Y. Recent Applications of nanomaterials in prosthodontics. J Nanomater 2015;1-11. [Doi. 10.1155/2015/408643].
2. Torres LS, Marin LM, Anita RE, Padron GH, Castano VM. Biocompatible metal-oxide nanoparticles: Nanotechnology improvement of conventional prosthetic acrylic resins. J Nanomater 2011;1-18. [Doi. 10.1155/2011/941561].
3. Gubin SP. Metal containing nano-particles within polymeric matrices: Preparation, structure, and properties. Colloids Surf A

Physicochem Eng Asp 2002;202:155-63.

4. Maki DG, Tambyah PA. Engineering out the risk for infection with urinary catheters. Emerg Infect Dis 2001;7:342-7.
5. Lansdown AB. Silver. I: Its antibacterial properties and mechanism of action. J Wound Care 2002;11:125-30.
6. Sawant SN, Selvaraj V, Prabhawathi V, Doble M. Antibiofilm properties of silver and gold incorporated PU, PCLm, PC and PMMA nanocomposites under two shear conditions. PLoS One 2013;8:e63311.
7. Corcione CE, Maffezzoli A. Glass transition in thermosetting claynanocomposite polyurethanes. Thermochim Acta 2009;485:43-8.
8. Maio ED, Iannace S, Sorrentino L, Nicolais L. Isothermal crystallization in PCL/clay nanocomposites investigated with thermal and rheometric methods. Polymer 2004;45:8893.
9. Sanchez-Soto M, Schiraldi DA, Illescas S. Study of the morphology and properties of melt-mixed polycarbonate-POSS nanocomposites. Eur Polymer J 2009;45:341-52.
10. Yeo SY, Jeong SH. Preparation and characterization of polypropylene/silver nanocomposite fibers. Polymer Int 2003;52:1053-57.
11. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO, *et al.* A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J Biomed Mater Res 2000;52:662-8.
12. Kraft CN, Hansis M, Arens S, Menger MD, Vollmar B. Striated muscle microvascular response to silver implants: A comparative *in vivo* study with titanium and stainless steel. J Biomed Mater Res 2000;49:192-9.
13. Gupta A, Silver S. Silver as a biocide: Will resistance become a problem? Nat Biotechnol 1998;16:888.
14. Lin CC, Yeh YC, Yang CY, Chen CL, Chen GF, Chen CC, *et al.* Selective binding of mannose-encapsulated gold nanoparticles to type 1 pili in *Escherichia coli*. J Am Chem Soc 2002;124:3508-9.
15. Khalili Fard J, Jafari S, Eghbal MA. A review of molecular mechanisms involved in toxicity of nanoparticles. Adv Pharm Bull 2015;5:447-54.
16. Dykman L, Khlebtsov N. Gold nanoparticles in biomedical applications: Recent advances and perspectives. Chem Soc Rev 2012;41:2256-82.
17. Tiwari PM, Vig K, Dennis VA, Singh SR. Functionalized gold nanoparticles and their biomedical applications. Nanomaterials (Basel) 2011;1:31-63.
18. Yeh YC, Creran B, Rotello VM. Gold nanoparticles: Preparation, properties, and applications in bionanotechnology. Nanoscale 2012;4:1871-80.
19. Iravani S. Green synthesis of metal nanoparticles using plants. Green Chem 2011;13:2638-50.
20. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, *et al*. Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, *Rhodococcus* species. Colloids Surf B 2003;28:313-8.
21. Shankar SS, Rai A, Ankamwar B, Singh A, Ahmad A, Sastry M, *et al.* Biological synthesis of triangular gold nanoprisms. Nat Mater 2004;3:482-8.
22. Ankamwar B, Damle C, Ahmad A, Sastry M. Biosynthesis of gold and silver nanoparticles using emblica officinalis fruit extract, their phase transfer and transmetallation in an organic solution. J Nanosci Nanotechnol 2005;5:1665-71.
23. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, *et al*. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinna-mommum camphora* leaf. Nanotechnology 2007;18:104-14.
24. Bera O, Pilic B, Pavlicevic J, Jovicic M, Hollo B, Mesaros Szecsenyi K, *et al*. Preparation and thermal properties of polystyrene/silica nanocomposites. Thermochimic Acta 2011;515:1-5.
25. Song JY, Jang HK, Kim BS. Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diospyros kaki* leaf extracts. Proc Biochem 2009;44:1133-38.
26. Nazirkar G, Bhanushali S, Singh S, Pattanaik B, Raj N. Effect of anatase titanium dioxide nanoparticles on the flexural strength of heat cured poly methyl methacrylate resins: An *in vitro* study. J Indian Prosthodont Soc 2014;14:144-9.
27. Jang DE, Lee JY, Jang HS, Lee JJ, Son MK. Color stability, water sorption and cytotoxicity of thermoplastic acrylic resin for non metal clasp denture. J Adv Prosthodont 2015;7:278-87.
28. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 2009;3:279-90.
29. Pan Y, Neuss S, Leifert A, Fischler M, Wen F, Simon U, *et al.* Size-dependent cytotoxicity of gold nanoparticles. Small 2007;3:1941-9.
30. Chithrani BD, Ghazani AA, Chan WC. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett 2006;6:662-8.
31. Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. Small 2005;1:325-7.
32. Pernodet N, Fang X, Sun Y, Bakhtina A, Ramakrishnan A, Sokolov J, *et al.* Adverse effects of citrate/gold nanoparticles on human dermal fibroblasts. Small 2006;2:766-73.
33. Roy N, Gaur A, Jain A, Bhattacharya S, Rani V. Green synthesis of silver nanoparticles: An approach to overcome toxicity. Environ Toxicol Pharmacol 2013;36:807-12.
34. Sodagar A, Bahador A, Khalil S, Shahroudi AS, Kassaee MZ. The effect of TiO2 and SiO2 nanoparticles on flexural strength of poly (methyl methacrylate) acrylic resins. J Prosthodont Res 2013;57:15-9.
35. Burunkova J, Denisiuk I, Vorzobova N, Daroczi L, Hegedus CS, Charnovych S, *et al.* Fabrication and characterization of gold/acrylic polymer nanocomposites. Eur Polymer J 2013;49:3072-7.
36. Xia Y, Zhang F, Xie H, Gu N. Nanoparticle-reinforced resin-based dental composites. J Dent 2008;36:450-5.
37. Ellakwa AE, Morsy MA, El-Sheikh AM. Effect of aluminum oxide addition on the flexural strength and thermal diffusivity of heat-polymerized acrylic resin. J Prosthodont 2008;17:439-44.
38. Fujishima M, Takatori H, Tada H. Interfacial chemical bonding effect on the photocatalytic activity of TiO2-SiO2 nanocoupling systems. J Colloid Interface Sci 2011;361:628-31.
39. Sodagar A, Kassaee MZ, Akhavan A, Javadi N, Arab S, Kharazifard MJ, *et al.* Effect of silver nano particles on flexural strength of acrylic resins. J Prosthodont Res 2012;56:120-4.
40. Chladek G, Kasperski J, Barszczewska-Rybarek I, Zmudzki J. Sorption, solubility, bond strength and hardness of denture soft lining incorporated with silver nanoparticles. Int J Mol Sci 2012;14:563-74.
41. Casemiro LA, Gomes Martins CH, Pires-de-Souza Fde C, Panzeri H. Antimicrobial and mechanical properties of acrylic resins with incorporated silver-zinc zeolite-part I. Gerodontology 2008;25:187-94.
42. Kassaee MZ, Akhavan A, Sheikh N, Sodagar A. Antibacterial effects of a new dental acrylic resin containing silver nanoparticles. J Appl Polym Sci 2008;110:1699-703.
43. Sehajpal SB, Sood VK. Effect of metal fillers on some physical properties of acrylic resin. J Prosthet Dent 1989;61:746-51.