



Synthesis and Characterization of Antifungal Nanocomposite AgSiO₂ Polymethyl Methacrylate

Mahmoud Sabouhi¹ Maryam Amini-Pozveh² Farshad Bajoghli³ Hamidreza Dastjerd⁴
Rasoul Mohammadi⁵

¹Department of Prosthodontics, Dental Materials Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

²Department of Prosthodontics Dentistry, Dental Materials Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

³Department of Prosthodontics, Dental Implants Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

⁴Institute of Biomaterial of Tehran University, Tehran University of Medical Science, Tehran, Iran

⁵Department of Medical Parasitology and Mycology, School of Medicine, Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Address for correspondence Maryam Amini-Pozveh, DDS, MS, Department of Prosthodontics Dentistry, Dental Materials Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran (e-mail: m.amini8412@gmail.com).

Eur J Dent 2022;16:109–114.

Abstract

Objectives Polymethyl methacrylate as the most common material used in denture bases has some problems. The aim of this study was to introduce a new nanocomposite of PMMA to improve flexural strength and antifungal properties.

Materials and Methods In this experimental study, AgSiO₂ nanoparticles were prepared, and their characteristics were confirmed by scanning electron microscope and energy dispersive spectroscopy techniques. Then the nanoparticles in the weight ratio of 0.1, 0.3, 0.5, and 0.7% were incorporated to heat-cured PMMA and the control group included no nanoparticles.

To measure the flexural strength before and after thermocycling three-point bending test was used. Eight samples per group with dimensions of 65 × 10 × 2.5 mm were used. Antifungal activity against *Candida albicans* (PTCC 5027) was investigated through colony count method. Statistical analysis was done by SPSS at significance level of *p*-value ≤ 0.05.

Results The mean flexural strength in groups 0.1, 0.3, and 0.7% was significantly higher than the control. After thermocycling flexural strength of the control group was significantly lower than 0.3 and 0.5% groups. As the concentration of nanoparticles increased the antifungal activity improved (*p* < 0.05).

Conclusion Addition of nanoparticles AgSiO₂ improved flexural strength and antifungal characteristics of PMMA.

Keywords

- ▶ antifungal
- ▶ *Candida albicans*
- ▶ colony count
- ▶ nanocomposite
- ▶ polymethyl methacrylate
- ▶ strength

published online
August 12, 2021

DOI <https://doi.org/10.1055/s-0041-1731831>
ISSN 1305-7456

© 2021. The Author(s).

This is an open access article published by Thieme under the terms of the Creative Commons Attribution License, permitting unrestricted use, distribution, and reproduction so long as the original work is properly cited. (<https://creativecommons.org/licenses/by/4.0/>).

Thieme Medical and Scientific Publishers Pvt. Ltd. A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Introduction

Since 1940¹ polymethyl methacrylate (PMMA) has been the most common material used in denture bases due to its favorable characteristics including esthetic and ease of use and repair; however, there are some unfavorable characteristics such as low fracture resistance^{2,3} and denture stomatitis.⁴ Denture fracture was one of the most common reasons for denture repair.^{2,3} Fractures happen more frequently in dentures since attachments limit the thickness of PMMA^{5,6} and therefore make it more prone to fracture. There are some ways to reinforce PMMA such as the addition of fibers.⁷⁻⁹ Further improvements have been made with nanoparticles.¹⁰⁻¹⁴

The flexural strength has a crucial role in acrylic resins resistance to forces which are induced in oral environment, so evaluation of flexural strength is considered as one of the most important characteristics of mechanical properties of acrylic resin.

In some studies, adding different nanoparticles improved the strength of PMMA¹¹⁻¹⁴ and in some others had adverse effect due to accumulation of nanoparticles around each other.^{11,14}

Thermocycling which happens in oral environment every day can decrease mechanical properties of PMMA. Therefore, it was considered in this study.^{15,16}

Another problem associated with denture wearer is *Candida* denture stomatitis, which is caused by patient conditions and characteristics of PMMA which is a suitable media for fungal growth.⁴

Different nanoparticles have proven to be antimicrobial agents such as AgSiO₂.^{17,18} But the effect of these antimicrobial agents on mechanical aspects of PMMA is not clear.

The goal of this study was to introduce a novel nanocomposite of PMMA with both antifungal activity and improved mechanical properties. Accordingly, AgSiO₂ nanoparticles were fabricated first and evaluated by physicochemical analysis. Then the nanocomposite of PMMA with different ratio of nanoparticles was fabricated and its antifungal (against *Candida albicans*) and strength characteristics were assessed.

The null hypothesis was that addition of AgSiO₂ to PMMA would not improve flexural strength and antifungal activity.

Material and Methods

In this experimental study first, AgSiO₂ nanoparticles were made, and added to four PMMA groups with proportions of 0.1, 0.3, 0.5, and 0.7%. In the control group no nanoparticles were added. Samples were evaluated for flexural strength and antifungal activity.

Synthesis of AgSiO₂ Nanoparticles

Sodium silicate liquid was diluted with distilled water with ratio of 1:4 and 1% of sodium dodecyl sulfate (SDS) was added. Temperature in this stage reached 90°C (PH of the solution was approximately 8 to 9 and stirring speed was 500 rpm). Temperature was lowered to 60°C and was stirred with speed of 200 rpm by a heat stirrer.

Sulfuric acid (concentration 98%) was diluted with distilled water in a ratio of 1:10 and based on stoichiometry the amount needed was provided. At this stage sulfuric acid solution was slowly added in 120 minutes to reach pH of 5.

Ten grams of 10% water solution of sodium chloride was added and stirred for 30 minutes. The heater was turned off and solution was gradually cooled while mixer was still working.¹⁹ After a while, nano-silica particles were deposited which were washed several times with distilled water to remove impurities and were placed in 200°C furnace to dry.

0.2 g silica nanoparticles, 1% SDS, and 0.1 g of silver nitrate were added in 50 mL of distilled water and stirred at 80°C.

The equal equivalents of silver nitrate and hydrazine were added. After 30 minutes of stirring, the solution was placed into the oven and dried at a temperature of 200°C. After cooling, nano-silver silica powder by weight portion of one silver, two silica was prepared.^{20,21}

Nanoparticles Characterization Test

The scanning electron microscope (SEM) (ZEISS, Germany) was used to study silver/silica nanoparticles and semiquantitative analysis of elements was done with energy dispersive spectroscopy (EDS).

The Development of Nanocomposites

Specimens Preparation and Measurement of Flexural Strength

Four aluminum models with dimensions of 65 × 10 × 2.5 mm were prepared and flaked. After setting of stone the flask was opened and aluminum dyes were removed and created space was replaced with heat-cured acrylic resin.

In the control group, heat-cured acrylic resin (Ivoclar, vivadent, Germany) was prepared according to manufacturing instructions and placed inside the empty space in the mold. After completion of polymerization, flask was opened slowly without stress. In this way acrylic resin samples were made.

In the case of nanocomposite samples, nanoparticle with the ratio of 0.1, 0.3, 0.5, and 0.7% weight for needed weight of acrylic resin was measured by digital scalar. In each group nanoparticles were dispersed by a magnetic stirrer into the acrylic resin monomer for 3 minutes, so even distribution of nanoparticles in resin matrix was achieved. The polymerization was done according to the manufacturer instructions. Samples were removed from the stone and thinned to appropriate dimensions by 450 grit sandpaper. Digital caliper was used to check the dimension of samples and those which were not appropriate were eliminated from the study. Samples were stored at 37°C for 50 hours.

The proper distribution of nanoparticles was investigated with SEM.

The flexural strength and deflection were measured for each group by using the loading machine (Walter testing machine, Germany) and with 3-point bending test and formula $S = 3pl/2bd^2$ in which S was flexural strength in Pascal, p was the amount of force exerted by the machine until breakage occurred in Newton, l was the distance between bases of

the machine where force was applied, b was specimen width, and d was specimen thickness all in meters.

Sample from each group which had flexural strength closest to the mean of that group was chosen for microscopic examination.

Preparation of Specimens for Thermocycling

Thermocycled samples were prepared by abovementioned method. Thermocycling was done with temperatures ranging between 55 and 5°C and 5,000 repetition. In this way samples were placed in 5°C water for 30 seconds and then out of water for 10 seconds and 30 seconds in 55°C water. After completion of 5,000 cycles, flexural strength and deflection were measured as previously.

Preparation of Specimens for Evaluation of Antifungal Activity

Specimens with size of 10 × 10 × 2.5 mm were prepared. The methods and groups were as mentioned before. To assess antifungal activity, *Candida albicans* (PTCC 5027) was added to Sabouraud dextrose agar (Difco, Detroit, Michigan, United States) and incubated at 37°C for 48 hours. A suspension of 10⁶ *albicans*/mL was made through spectrophotometer (Biowave Biochrom, England) and wavelength of 530 nm.

Specimens were sterilized with UV waves for 5 minutes and then entered in a sterile tube containing 2 mL of fungal suspension. After incubation period of 90 minutes in 37°C the specimens were removed and were rinsed three times with distilled water for 1 minute. Then each specimen was entered

in the tube containing 10-mL sterile saline and the adhered cells entered the saline by means of vortexing (Vortex mixer, United States). From this suspension, dilutions of 10⁻¹, 10⁻², and 10⁻³ in sterile saline were made. Then 0.1 mL of prepared suspension was entered into SDA medium and incubated at 37°C for 48 hours. The microorganisms were counted in CFU/mL.²²

Statistical analysis was done with SPSS software version 23 and by one-way ANOVA and Kruskal–Wallis test.

Results

Evaluation of AgSiO₂ Nanoparticle

The AgSiO₂ nanoparticles were examined, and their size and quality were verified by SEM and EDS method. SEM images showed size of nanoparticles in the average range of 20 and 25 nm (► Fig. 1).

EDS evaluation (in Kv 10.0, take off angle 35.0 degrees, and elapsed life time 30.0ms) of the three areas revealed the Homogen distribution of silver and silica nanoparticles. Point check of nanoparticles showed the purity of silver/silica nanoparticles over 98% (► Fig. 2).

Nanocomposites Microscopic Examination

SEM images showed the distribution of silver/silica nanoparticles in acrylic resin which impacted on mechanical and other properties of acrylic resin (► Fig. 3).

The results of flexural strength and antifungal activity have been mentioned in the following.

Results of Flexural Strength

One-way ANOVA and Tukey HSD analysis showed significant differences between the control group and 0.5% group with the other groups in such a way that the flexural strength of 0.1, 0.3, and 0.7% group was significantly higher than the control group and 0.5% group of the nanoparticles (p -value <0.001). However, there was no significant difference between the control and 0.5% (p -value = 0.07). The results of flexural strength are shown in ► Fig. 4.

Results of Flexural Strength after Thermocycling

Flexural strength of the control group after thermocycling was lower than the other groups. One-way ANOVA and Tukey HSD analysis showed this difference was statistically significant in 0.3% (p -value = 0.003) and 0.5% (p -value = 0.03) groups. The results of flexural strength after thermocycling are shown in ► Fig. 5.

Results of Antifungal Activity

One-way ANOVA and Mann-Whitney test revealed as the percent of nanoparticles increased the fungal colony count significantly decreased (p -value = 0.05). The results of antifungal activity are shown in ► Fig. 6.

Discussion

As the elderly population is increasing there is an increase in need for dental prostheses. PMMA is one of the most widely

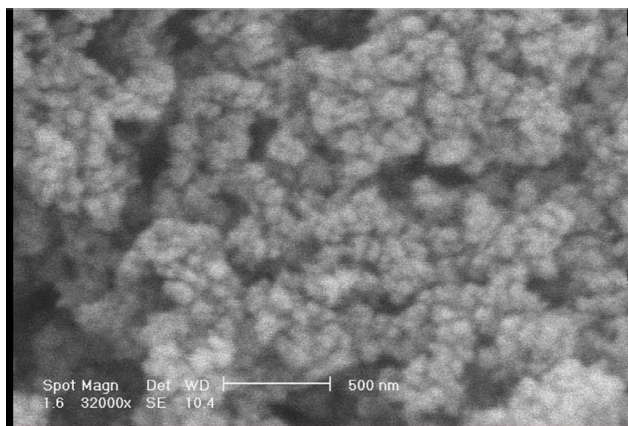


Fig. 1 SEM illustration of AgSiO₂ nanoparticles. SEM, scanning electron microscope.

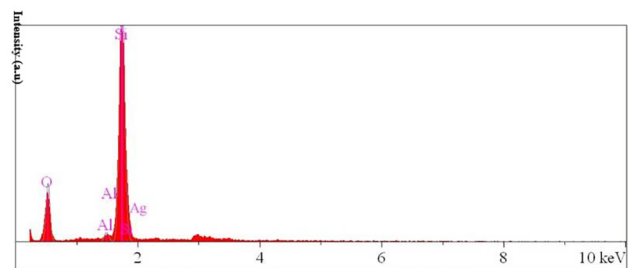


Fig. 2 EDS illustration of AgSiO₂ nanoparticles. EDS, energy dispersive spectroscopy.

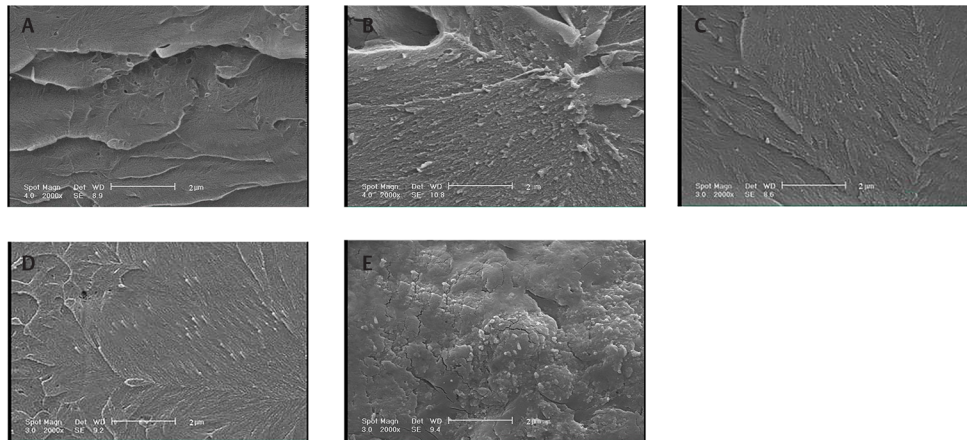


Fig. 3 (A) SEM illustration of acrylic resin. (B) SEM illustration of distribution of silver/silica nanoparticles in acrylic resin in group 0.1% nanoparticle. (C) SEM illustration of distribution of silver/silica nanoparticles in acrylic resin in group 0.3% nanoparticle. (D) SEM illustration of distribution of silver/silica nanoparticles in acrylic resin in group 0.5% nanoparticle. (E) SEM illustration of distribution of silver/silica nanoparticles in acrylic resin in group 0.7% nanoparticle. SEM, scanning electron microscope.

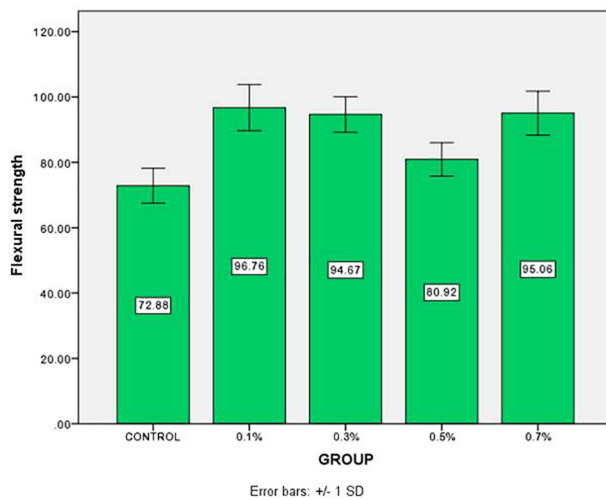


Fig. 4 Profile of flexural strength.

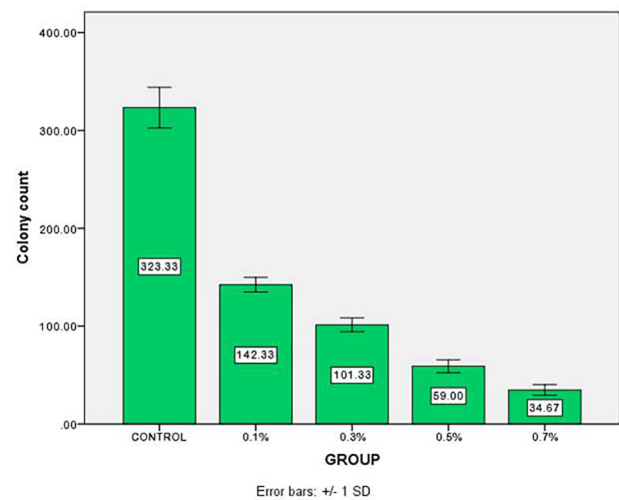


Fig. 6 Profile of colony count.

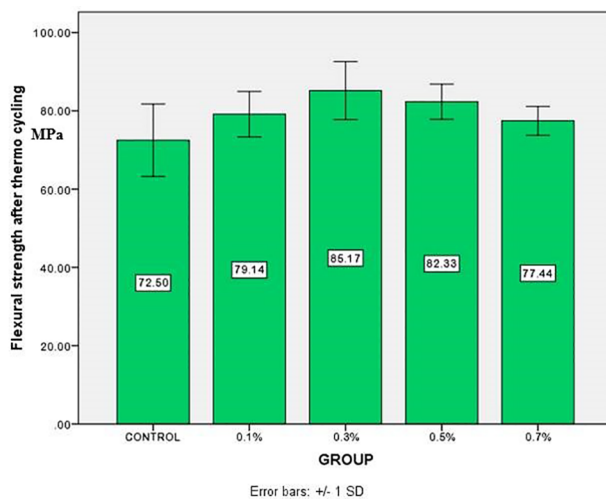


Fig. 5 Profile of flexural strength after thermocycling.

during function.² Therefore, there is a need to improve its strength. Improvement in nanotechnology has led its way to the field of dentistry. In this study, it was tried to improve characteristics of PMMA by introducing a new nanocomposite. The hypothesis that addition of AgSiO₂ to PMMA would not improve flexural strength and antifungal activity was rejected based on the results.

Although patient hygiene has an important role in the inhibition of denture stomatitis, but in very old or immune-deficient patients there is a need to inhibit microorganism growth. It has been proven that nanotechnology can provide antimicrobial effects.^{17,18} It was important to use a nanoparticle with antimicrobial effect without disadvantage for mechanical properties. Since the flexural strength has an important role in acrylic resin resistance against forces exerted on prosthesis in the mouth, evaluation of flexural strength is one of the most important factors in acrylic resins. Therefore, in this study flexural strength was chosen to measure the mechanical properties.

To use or not to use other auxiliary factors to bond nanoparticles with acrylic resins was another issue that

used materials in fabrication of removable prosthesis.¹ The most common problem with it is its fracture

had to be decided upon. In some studies, adding coupling agents to improve more effective bond between the filler and acrylic resin was emphasized. For example, in a study adding only 0.1% of the coupling agent showed favorable results on mechanical properties, but more concentrations resulted in poor mechanical properties. However, the effect of humidity on the bond strength in long term was not considered.²³

A review article on silane coupling agents reported that it will eventually disintegrate in the mouth environment.²⁴

In another study the relationship between the amount of silane on filler and the weakening effect of mechanical properties after immersion in water was demonstrated.²⁵

In this study, nanoparticles were conditioned with methacrylic acid which is one of the ingredients of heat-cured PMMA monomer, as a coupling agent. This method was used by Khaled et al²⁶ for synthesis of TiO₂-PMMA nanocomposite. So, the nanoparticles were ready to bond with polymer matrix. With this method successful bond between nanoparticles and polymer matrix was made without disadvantages of external silanes.

In some studies, addition of nanoparticles improved flexural strength of the acrylic resin¹¹⁻¹⁴ and in some others, it had negative effects due to the accumulation of nanoparticles around each other.^{11,14} Even distribution of the nanoparticles in acrylic resin, depends on stirring rate, period of stirring, and temperature. The more uniform distribution of nanoparticle causes more uniform physical, mechanical, and biological properties. In this study magnetic stirrer as an available specified device was used for a certain time to decrease nanoparticles aggregation and provide conditioning and dispersion of nanoparticles in the monomer.

From each group, the sample which was closest to the average flexural strength of that group was selected to be studied microscopically by electron microscopy to have better interpretation of the observed properties. In this study, addition of nanoparticles improved flexural strength, which was statistically significant in groups 0.1, 0.3, and 0.7%. The SEM investigation showed that in 0.1 and 0.3% groups light distribution of nanoparticles was obtained but in 0.5 group accumulation of nanoparticles was observed. In 0.7% group heavy but even distribution of nanoparticles was observed. This distribution explained why in 0.5% group the strength was reduced.

Thermocycling can be expected in the stressful environment of the mouth and happened in everyday life. In previous studies the negative effect of this phenomenon on acrylic resin has been reported.^{15,16} In the present study, flexural strength improved by adding nanoparticles even after thermocycling in all groups, but this improvement was statistically significant in 0.3 and 0.5% group compared with the control group. It seems that thermocycling can degrade polymer's bond, so it is probable that in 0.7% group more bonds were degraded than others with lower bond counts. In 0.1% group less amount of bond was formed from the beginning and from this amount, some were degraded due to thermocycling stress.

Another area to consider is the effect of addition of these nanoparticles on color of samples. Since AgSiO₂ nanoparticles

had relatively dark shade in acrylic resins, especially in the 0.7% group, if the main purpose is to improve mechanical properties, it may be better to use nanoparticle in lower concentrations and if there is a need for most antifungal activity, dark shade should be covered with some proper pigments.

The limitation of this study was performing it in the experimental situation. Also, it is suggested to evaluate biocompatibility and some other mechanical properties in another study.

Conclusion

Addition of nanoparticles AgSiO₂ to PMMA led to an increase in flexural strength; the best results were seen in 0.1, 0.3, and 0.7% nanoparticles groups.

Addition of nanoparticles AgSiO₂ to PMMA led to increase in flexural strength after thermocycling and the best results were seen when 0.3 and 0.5% nanoparticles were added.

Addition of AgSiO₂ improved antifungal activity significantly.

Authors' Contributions

M.S. was the supervisor of the project. H.D. made the nanoparticle and did the laboratory tests. F.B. was the advisor of project and edited the article. M.A.-P. made the sample and wrote the main text. R.M. was the advisor of project and did the microbial tests.

Funding

This study was supported by Isfahan University of Medical Sciences (research grant 395333).

Conflict of Interest

None declared.

Acknowledgment

This article was based on a thesis submitted to the School of Dentistry, Isfahan University of Medical Sciences in partial fulfillment of the requirement for the MSc degree.

References

- 1 Krunic N, Kostic M, Andelkovic M. Acrylic resins: still irreplaceable materials in prosthetic dentistry. *Acta Stomatol Naissi* 2007;23(56):749-754
- 2 Choudhary S. Complete denture fracture—a proposed classification system and its incidence in National Capital Region population: a survey. *J Indian Prosthodont Soc* 2019;19(4):307-312
- 3 Rajesh P, Ganapathy D, Chaudhary M. Prevalence of complete denture fracture—a retrospective study. *PalArch's J Archaeol Egypt* 2020;17(7):502-508
- 4 Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. *J Prosthodont* 2011;20(4):251-260
- 5 Gonda T, Ikebe K, Dong J, Nokubi T. Effect of reinforcement on overdenture strain. *J Dent Res* 2007;86(7):667-671
- 6 Gonda T, Maeda Y, Walton JN, MacEntee MI. Fracture incidence in mandibular overdentures retained by one or two implants. *J Prosthet Dent* 2010;103(3):178-181
- 7 Hussain WA, Hashim FS. Effect of additives on impact strength of denture base resin. *Iraqi J Sci* 2017;58(2B):860-867
- 8 Demir H, Görler O, Doğan A, Ozden S. The assessment of impact properties of a denture base polymer reinforced with various fillers. *Int J Acad Res* 2017;9(1):15-19

- 9 Golbidi F, Pozveh MA. Flexural strength of polymethyl methacrylate repaired with fiberglass. *J Dent (Tehran)* 2017;14(4):231–236
- 10 Wang R, Kayacan R, Küçükeşmen C. Nanotubes/polymethyl methacrylate composite resins as denture base materials. In: Zhang M, Naik RR, Dai L, eds. *Carbon Nanomaterials for Biomedical Applications* Springer International; 2016; 227–240
- 11 Wang R, Tao J, Yu B, Dai L. Characterization of multiwalled carbon nanotube-polymethyl methacrylate composite resins as denture base materials. *J Prosthet Dent* 2014;111(4):318–326
- 12 Ahmed MA, Ebrahim MI. Effect of zirconium oxide nano-fillers addition on the flexural strength, fracture toughness, and hardness of heat-polymerized acrylic resin. *World J Nano Sci Eng* 2014;4:50–57
- 13 Alnamel HA, Mudhaffer M. The effect of silicon di oxide nano-fillers reinforcement on some properties of heat cure polymethyl methacrylate denture base material. *J Baghdad Coll Dent* 2014;26(1):32–36
- 14 Pan Y, et al. Novel acrylic resin denture base with enhanced mechanical properties by the incorporation of PMMA-modified hydroxyapatite. *Prog Nat Sci* 2013;23(1):89–93
- 15 Sasaki H, Hamanaka I, Takahashi Y, Kawaguchi T. Effect of long-term water immersion or thermal shock on mechanical properties of high-impact acrylic denture base resins. *Dent Mater J* 2016;35(2):204–209
- 16 Takahashi Y, Hamanaka I, Shimizu H. Effect of thermal shock on mechanical properties of injection-molded thermoplastic denture base resins. *Acta Odontol Scand* 2012;70(4):297–302
- 17 Kim JS, Kuk E, Yu KN, et al. Antimicrobial effects of silver nanoparticles. *Nanomedicine (Lond)* 2007;3(1):95–101
- 18 Dhanalekshmi KI, Meena KS. Comparison of antibacterial activities of Ag@TiO₂ and Ag@SiO₂ core-shell nanoparticles. *Spectrochim Acta A Mol Biomol Spectrosc* 2014;128:887–890
- 19 Musić S, Filipović-Vinceković N, Sekovanić L. Precipitation of amorphous SiO₂ particles and their properties. *Braz J Chem Eng* 2011;28(1):89–94
- 20 Han Q, Li G, Wang D, et al. Synthesis of Ag-SiO₂ composite nanospheres and their catalytic activity. *Sci China Chem* 2014;57(6):881–887
- 21 Kim YH, Lee DK, Kang YS. Synthesis and characterization of Ag and Ag-SiO₂ nanoparticles. *Colloids Surf A Physicochem Eng Asp* 2005;257:273–276
- 22 Cierech M, Kolenda A, Grudniak AM, et al. Significance of polymethylmethacrylate (PMMA) modification by zinc oxide nanoparticles for fungal biofilm formation. *Int J Pharm* 2016;510(1):323–335
- 23 Chajareenont P, Takahashi H, Nishiyama N, Arksornnukit M. Effect of different amounts of 3-methacryloxypropyltrimethoxysilane on the flexural properties and wear resistance of alumina reinforced PMMA. *Dent Mater J* 2012;31(4):623–628
- 24 Lung CYK, Matinlinna JP. Aspects of silane coupling agents and surface conditioning in dentistry: an overview. *Dent Mater* 2012;28(5):467–477
- 25 Debnath S, Wunder SL, McCool JI, Baran GR. Silane treatment effects on glass/resin interfacial shear strengths. *Dent Mater* 2003;19(5):441–448
- 26 Khaled SM, Sui R, Charpentier PA, Rizkalla AS. Synthesis of TiO₂-PMMA nanocomposite: using methacrylic acid as a coupling agent. *Langmuir* 2007;23(7):3988–3995