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# The Effect of Magnesium Oxide (MgO) Nano-Fillers on the Antibacterial Activity and Some Properties of Heat Cured Acrylic Resin

**Thoalnorain A. Shakir, B.D.S.[1], Shorouq M. Abass, B.D.S., M.Sc.[2]**

[1]Master Student. Department of Prosthodontics, College of Dentistry, University of Baghdad

[2]Assistant Professor, Department of Prosthodontics, College of Dentistry, University of Baghdad

**Abstract:** ***Background:*** ***Polymethyl methacrylate (PMMA) resins were mostly used in fabrication of removable dentures. They supports microbial colonization and biofilm formation on its surface, which is a crucial step for various oral infections. The aim of the current study was to investigate the effect of MgO nanoparticles by using incorporation and immersion technique at different duration and percentages on the Staphylococcus aureus colony forming unites (CFU) and some properties of heat cured acrylic denture base material.*** ***Materials and methods:*** ***two techniques (immersion and incorporation) were used to introduce nanosized MgO powder to heat cured acrylic resin. MgO nanoparticles solution was prepared from the addition of 7% of unmodified nanoparticles by weight to 50 ml of normal saline. 1.5% of MgO nano fillers were incorporated into heat cured acrylic to obtain PMMA/ MgO nanocomposite. The antibacterial activity of 7% MgO nanoparticles aqueous solution was evaluated at two different periods (10 and 30 minutes) by using colony forming unites (CFU) of Staphylococcus aureus. The impact strength of study specimens was measured by Charpy impact testing machine. Three point bending approach was performed to evaluate the transverse strength of test specimens. Surface roughness was conducted by profilometer device (portable roughness tester).*** ***Results:*** ***the results of this study showed that the immersion of acrylic specimens in 7% MgO nanoparticles solution for different periods led to a highly significant reduction in colony forming unites (CFU) of Staphylococcus aureus in comparison with the control group. In addition, a non-significant difference in impact strength, transverse strength and surface roughness was obtained by 30 minutes immersion in that solution, while a highly significant difference was resulted from the incorporation of 1.5% of unmodified MgO nano particles when compared with control group.*** ***Conclusion:*** ***immersion in 7% MgO nanoparticles solution was significantly effective in reducing the growth of Staphylococcus aureus on heat cured acrylic resin, with insignificant effect on the impact strength, transverse strength and surface roughness.***

**Keywords:** MgO Nano particles, heat cured acrylic, *Staphylococcus aureus*

## 1. Introduction

Polymethyl methacrylate (PMMA) resins were mostly used in fabrication of removable dentures. The popularity and universal use of PMMA based resin as a denture base material comes from its ease of processing and reparability, low coast, lightweight, biocompatibility, low water solubility and sorption, excellent aesthetic appearance [1] and accuracy of fit [2]. However, it still far from fulfilling all the criteria of ideal denture base material and have several drawbacks such as the susceptibility to microbial colonization and biofilm formation on its surface making the denture a harbor of microorganisms and source of various infections [3]. Moreover, effective biofilm removal requires a degree of manual dexterity that is often lacking among older individuals [4]. Over the years, many researches have focused on ways to improve the antimicrobial activity of PMMA based rein via surface and chemical modification. These studies led to development of new denture polymers with suitable mechanical and physical properties that include anionic PMMA polymers [5]. While, a number of studies have been conducted to establish an intra-oral drug delivery system through the incorporation of certain antifungal or antimicrobial agents into acrylic resin [6]. Metal oxide nanoparticles have been extensively used in different industrial and biomedical applications due to their unique properties [7]. Among the most known nanometals, much attention have been focused on magnesium oxide nanoparticles because of their antibacterial activity against various pathogens [8, 9]. The present study was conducted to investigate the effect of incorporation and immersion MgO nanoparticles at different percentage and durations on the colony forming unites (CFU) of *Staphylococcus aureus* and also on the impact strength, transverse strength and surface roughness of heat cured acrylic denture base material.

## 2. Materials and Methods

One hundred thirty (130) specimens were fabricated to be used in this study. 40 specimens were prepared from heat cured acrylic to evaluate the antibacterial activity of 7% MgO nanoparticles aqueous solution in two different periods (10 and 30 minutes) and divided into 4 groups, 2 groups for each selected period. Impact strength, transverse strength and surface roughness were then evaluated by the construction of 90 specimens, which were divided into three groups: control group, immersion in 7% MgO nanoparticles solution for 30 minutes before testing procedure. In addition to, the third group, which was prepared from the incorporation of 1.5% of unmodified MgO nano fillers into PMMA based resin. Each one of these three groups was furtherly divided into 3 subgroups according to the test type.

**Selection of Proper Percentage of MgO nanoparticles**

According to the results of pilot study, the incorporation of different percentages of modified and unmodified MgO nano fillers had no action on the antibacterial activity of heat-cured acrylic resin. For this reason, a second pilot study was

conducted to evaluate the antibacterial activity of magnesium oxide nanoparticles (MgO NPs) solution against colonized bacteria onto acrylic specimens after immersion at different time intervals (10 and 30 minutes). It revealed that 7wt% MgO NPs solution was more effective in reducing the colony forming unites (CFU) of staphylococcus aureus. While the results of pilot study on other properties of heat-cured acrylic showed that the addition of 1.5wt% of unmodified MgO nano fillers led to an acceptable decrease in the value of impact strength when compared with other concentrations.

**Test specimens preparation**

Three different plastic patterns were constructed according to the performed test (Figure 1):

1) Antibacterial activity test: a disk shaped specimen with dimensions of 5mm in diameter and 2mm in thickness according to the dimensions of test tubes used in testing procedure.
2) Impact strength test: a bar shaped specimen with dimensions of 80mm in length, 10mm in width and 4mm in thickness [10].
3) Transverse strength test and surface roughness test: a bar shaped specimen with dimensions of 65mm in length, 10mm in width and 2.5mm in thickness [11].


**Figure 1:** plastic models used in the study; A for Impact strength, B for transverse strength specimen and C for antibacterial activity test specimens

**Mold preparation**

The conventional flasking technique was followed to prepare the molds of study specimens.

**Proportioning and mixing of PMMA**

**Table 1:** Proportioning and mixing of PMMA

| Percentage of MgO nano fillers | Amount of MgO nano fillers | Amount of PMMA powder | Amount of PMMA liquid |
|---|---|---|---|
| 0% (controlled group) | 0 g | 50 g | 21.7 ml |
| 1.5% | 0.75 g | 49.25 g | 21.7 ml |

**Addition of magnesium oxide (MgO) Nano fillers**

MgO nano fillers were added to PMMA monomer, Solution mixing was done by Ultrasonic disintegrator in parameters of (60 KHz, 120 W and 3 minutes) to provide good nano fillers' dispersion and avoid their aggregation within the monomer [3] (figure 2). The resin powder was then promptly blended with nano suspension to diminish the likelihood of Nano fillers' aggregation and separation.


**Figure 2:** A, B nanofillers' dispersion within the monomer by Ultrasonic disintegrator

**Specimens grouping**

Specimens grouping and sampling of the present study were shown in Table 2

**Table 2:** specimens grouping and sampling of the main study

| Group's symbol | Percentage of MgO nano fillers | Type of MgO treatment | Number of specimens | Test name |
|---|---|---|---|---|
| A | 0% | No treatment | 10 | Impact strength, transverse strength and surface roughness |
| B 10 | 7% | Immersion in MgO NPs solution for 10 minutes | 10 | Antibacterial activity |
| B 30 | 7% | Immersion in MgO NPs solution for 30 minutes | 10 | Antibacterial activity, Impact strength, transverse strength and surface roughness |
| C 10 | 0% | Immersion in normal saline for 10 minutes | 10 | Antibacterial activity |
| C 30 | 0% | Immersion in normal saline for 30 minutes | 10 | Antibacterial activity |
| D | 1.5% | Incorporation | 10 | Impact strength, transverse strength and surface roughness |

**Pre-test period**

All study specimens were stored in distilled water and incubated at 37°C for 48 hours prior to testing [12].

**Antibacterial activity test**

**Isolation of Staphylococcus aureus**

Fifteen isolates of *staphylococcus aureus* were obtained from 58 samples that taken from patients' throat and saliva by using sterile cotton swabs, and then inoculated into selective media for *staphylococcus aureus* growth such as Mannitol salt agar (which was prepared according to manufacturer's instructions). The agar plates were incubated at 37°C for 48 hours under aerobic condition.

**Identification of Staphylococcus aureus:**

Mannitol-fermenting Yellowish circular colonies of *staphylococcus aureus* were subcultured on blood agar (Figure 3) (which was prepared according to manufacturer's instructions) and incubated for 24 h at 37°C to confirm the identification by performing Coagulase, Catalase test and gram's stain method. Furthermore, antimicrobial susceptibility profile of the isolated strains was determined by disk diffusion method to differentiate the multidrug resistance from other *staphylococcus aureus* strains.

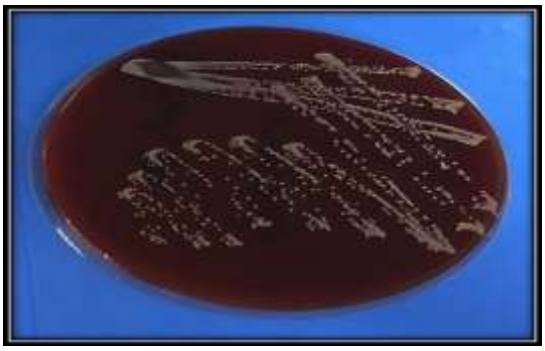

**Figure 3:** growth of *staphylococcus aureus* on blood agar medium

**Evaluation the antibacterial activity of MgO NPs solution:**

MgO NPs solution was prepared from the addition of 7% of unmodified nanoparticles by weight to 50 ml of normal saline. Solution mixing was done by Ultrasonic disintegrator in parameters of (60 KHz, 120 W and 3 minutes) to provide good dispersion of MgO nanoparticles and avoid their aggregation within the normal saline. Isolated *staphylococcus aureus* colonies were transferred to 10 ml sterile normal saline to achieve a bacterial suspension of turbidity equivalent to 0.5 McFarland standard (106 CFU/ml). 100 µl of the prepared bacterial suspension was inoculated into 9 ml of brain-heart infusion broth under sterile condition and sterile acrylic specimens were placed individually into the inoculated tubes and incubated at 37ºc for one hour under aerobic condition. Then the acrylic specimens were immersed in the previously prepared denture cleanser, in addition to 50 ml of sterile normal saline that was selected to be a positive control for 10 minutes and 30 minutes under Ultrasonic waves of 50 Hz frequency to avoid the aggregation of nanoparticles within the antiseptic solution (figure 4). After that, each specimen was placed in a test tube contained 5 ml of distilled water and shacked by autovortex mixer for 2 minutes to allow releasing of bacteria from specimen surface, then 20µl had been taken from the suspension to be streaked on blood agar plate. After 24 h incubation for the plates, the final count (number of bacteria per milliliter) was calculated in the following equation:

$$(CFU/ml) = \frac{\text{Colony number X Dilution}}{100} \quad [13]$$

**Figure 4:** disinfectant treatment of acrylic specimens under ultrasonic waves

**Impact strength test**

It was carried out by charpy impact testing machine (Testing Machines Inc. New York, U.S.A.) (figure 8) according to ISO 179-1 (2000).

**Transverse strength test**

Instron universal testing machine (Instron 1195 tester, UK) was used to evaluate the transverse strength of test specimens by three-point bending approach.

**Surface roughness test**

Portable roughness tester (TR200, Time Group Inc., Beijing, China) was used to evaluate the impact of MgO NPs on acrylic micro geometry. Three area were scanned for each specimen and the surface roughness was obtained from the mean value of these three readings.

## 3. Results

The immersion of acrylic specimens in 7% MgO NPs solution for different periods (group B 10, B 30) showed a highly significant reduction in colony forming unites of *staphylococcus aureus* in comparison to control group (C 10, C 30) (Table 3 and 4, Figure 5). In addition, a non-significant difference in impact strength (Table 5 and 6, Figure 6), transverse strength (Table 7 and 8, Figure 7) and surface roughness (Table 9 and 10, Figure 8) was obtained from the immersion of acrylic specimens in 7% MgO NPs solution for 30 minutes, while a highly significant difference was resulted from the incorporation of 1.5% of unmodified MgO nano fillers when compared with control group (A).

**Table 3:** descriptive analysis and ANOVA test for colony forming unites of *staphylococcus aureus.*

| Test Group | No. | Min | Max | Mean | Std. | F test | *P value |
|---|---|---|---|---|---|---|---|
| B30 | 10 | .018 | .020 | .0188 | .0007 | 1483.604 | 0.000 |
| B10 | 10 | .019 | .021 | .0202 | .0009 | | |
| C30 | 10 | .00 | .0001 | .0003 | .0004 | | |
| C10 | 10 | .010 | .012 | .0111 | .0007 | | |

**P < 0.01 Highly Significant*

**Table 4:** Bonferroni test between mean values of colony forming unites of staphylococcus aureus.

| Test Group | Mean Difference | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| C30 - C 10 | -.01080* | .000 | -.0117 | -.0099 |
| C30 - B 30 | -.01850* | .000 | -.0194 | -.0176 |
| C30 - B 10 | -.01990* | .000 | -.0208 | -.0190 |
| C 10 - B 30 | -.00770* | .000 | -.0086 | -.0068 |
| C 10 - B 10 | -.00910* | .000 | -.0100 | -.0082 |
| B 30 - B 10 | -.00140* | .001 | -.0023 | -.0005 |

**P < 0.01 Highly Significant.*

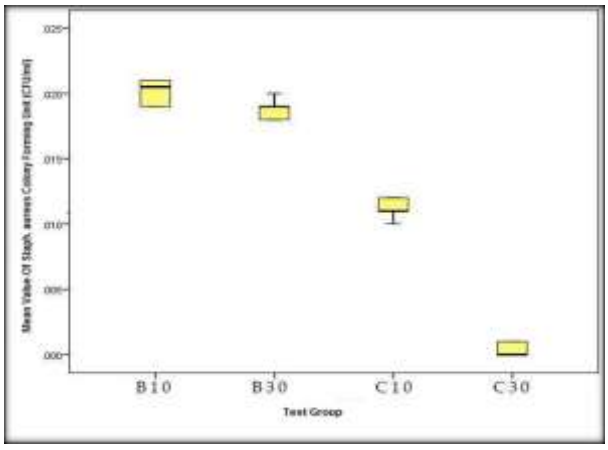


**Figure 5***:* mean values of colony forming unites of Staphylococcus aureus

**Table 5:** Descriptive analysis and ANOVA test for impact strength test

| Test Group | No. | Min | Max | Mean | Std. | F test | *P value |
|---|---|---|---|---|---|---|---|
| A | 10 | 8.37 | 13.59 | 11.40 | 1.54 | 1483.604 | 0.000 |
| C 30 | 10 | 8.12 | 13.58 | 11.19 | 1.57 | | |
| D | 10 | 7.09 | 9.57 | 8.05 | 0.88 | | |

**P < 0.01 Highly Significant.*

**Table 6:** Multiple Comparisons (Bonferroni) between mean values of impact strength

| Test Group | Mean Difference | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| A – D | 3.14445* | 0 | 1.5755 | 4.7134 |
| A -C 30 | -0.21068 | 1 | -1.7796 | 1.3583 |
| D - C 30 | -3.35513* | 0 | -4.9241 | -1.7862 |

**P < 0.01 Highly Significant*

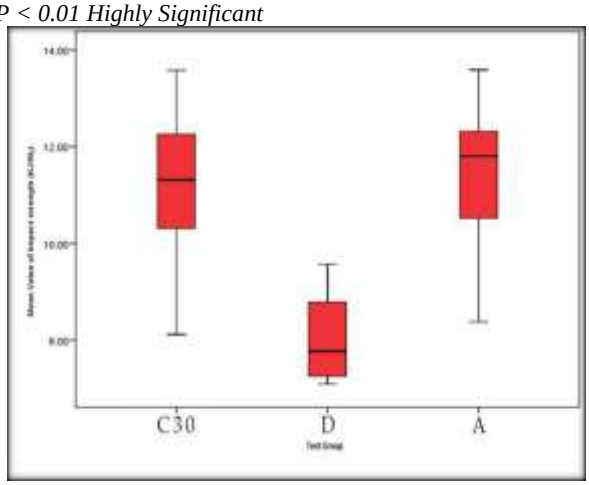


**Figure 6**: mean values of impact strength test

**Table 7:** Descriptive Statistics and ANOVA test for mean values of transverse strength.

| Test Group | No. | Min | Max | Mean | Std. D | F-test | *P value |
|---|---|---|---|---|---|---|---|
| A | 10 | 96 | 106.2 | 101.3 | 3.13 | 18.693 | 0.000 |
| D | 10 | 78.7 | 96.3 | 86.2 | 4.84 | | |
| C 30 | 10 | 97.9 | 103.9 | 101.3 | 1.94 | | |

**P < 0.01 Highly Significant.*

**Table 8:** multiple comparison test (Bonferroni test) between mean values of Transverse strength

| Test Group | Mean Difference | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| A – D | 15.15211* | .000 | 11.13 | 19.16 |
| A - C 30 | .06139 | 1.000 | -3.95 | 4.07 |
| D - C 30 | -15.09073* | .000 | -19.10 | -11.07 |

**P < 0.01 Highly Significant*

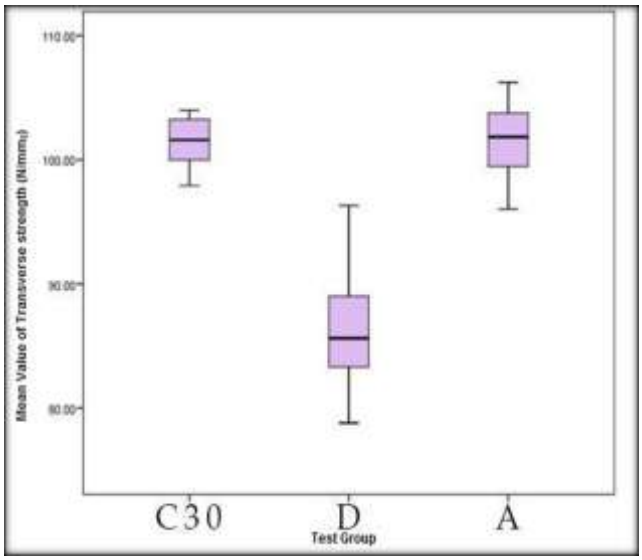


**Figure 7:** mean values of transverse strength

**Table 9:** Descriptive Statistics and ANOVA test for mean values of surface roughness

**P < 0.01 Highly Significant.*

| Test group | No. | Min | Max | Mean | Std. | F- test | P value |
|---|---|---|---|---|---|---|---|
| A | 10 | 0.49 | 1.48 | 0.941 | 0.27 | 14.643 | 0.000 |
| C 30 | 10 | 0.39 | 1.50 | 0.963 | 0.41 | | |
| D | 10 | 1.27 | 1.93 | 1.608 | 0.21 | | |

**Table 10:** multiple comparison (Bonferroni) between mean values of surface roughness

| Test Group | Mean Difference | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| A – D | -.64440* | 0.000 | -1.0015 | -0.2873 |
| A - C 30 | 0.02217 | 1.000 | -0.3350 | 0.3793 |
| D - C 30 | .66657* | 0.000 | 0.3094 | 1.0237 |

**P < 0.01 Highly Significant*

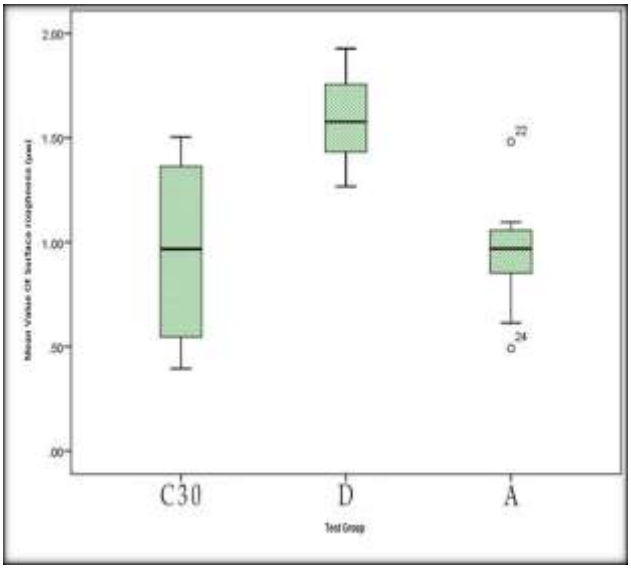


**Figure 8:** mean values of surface roughness

## 4. Discussion

The present study showed a highly significant reduction in colony forming unit of staphylococcus aureus after immersion the acrylic specimens in 7% MgO NPs solution at different intervals (10 and 30 minutes). It also revealed that the antibacterial activity of this solution was furtherly enhanced with increasing immersion time. This reduction in the viability of Staphylococcus aureus may be related to the antibacterial activity of nanoscale MgO particles. Generally, several studies have showed that the antimicrobial behavior of MgO NPs may be dependent on its surface area which determines the surface toxicity of nanoparticles [14], particle size where the antibacterial activity of MgO nanoparticles increases with reduced particle size [15], amount of nanoparticles [16], bacterial strain where MgO nanoparticles exhibit greater toxicity against gram-positive bacteria due to thin cellular wall of microorganism and its negative charge that helps to attract the nanoparticles [17] and exposure time to nanoparticles [18]. These findings were agreed with those of Silva et al., 2013 [19] study which revealed that silver nanoparticle colloidal solution significantly reduced the growth and biofilm forming ability for both of candida albicans and candida glabrata on the surface of heat-cured acrylic specimens. The absence of antibacterial activity of PMMA/ MgO nanocomposite in our investigation may be associated with poor release of superoxide anions from the incorporated MgO NPs and also probably due to inability of nanoparticles within PMMA specimen to be in direct contact with the colonogenic microorganisms on acrylic surface. This confirmed an agreement with Wady et al. (2012) [20]. While, it disagrees with Cierech et al. (2016) [21]. This might be due to different preparation procedure of Polymer/Nano Composite. Test specimens of PMMA/ MgO nanocomposite exhibited a highly significant decrease in the values of impact strength in comparison to control specimens. This reduction may be resulted from the brittleness of MgO nanoparticles and their inability to resist the impact loading [22]. It may also be due to Van der walls force of interfacial adhesion between inorganic nano fillers and PMMA matrix. These weak bonds will reduce the elastic modulus of modified polymer at lower stresses but tend to break at high degree of impact loading which will make the material more brittle (decease the toughness). MgO nanoparticles may act as stress concentration centers that facilitate the propagation of cracks through acrylic specimen [3]. Our consumptions were in agreement with Asopa et al. (2015) [23]. However, these results disagree with Dahham (2014) [12]. On the other hand, the immersion of acrylic specimens in 7% MgO NPs solution for 30 minutes didn't have any significant effect on impact strength of heat cured resin. We found that the incorporation of 1.5% of unmodified MgO nano fillers led to a highly significant decrease in the transverse strength of heat-cured acrylic. Its explanation could be associated with the reduction in cross-section of load bearing area of PMMA matrix [24]. It may also be due to the location of MgO nanoparticles between PMMA chains, which may restrict the mobility of these chains and their deformation ability during testing procedure [25]. Since MgO nano fillers have thermal insulation properties [26], they may reduce the conduction of heat during PMMA curing, in addition to the adverse relationship between the degree of nanofiller dispersion and the level of reacted monomer [27]. These factors may lead to an increase in the amount of residual monomer and formation of voids. Similar results were conducted by Akkuş et al. (2015) [28]. Whereas, different results were obtained by Dahham (2014) [12]. It can be explained by saying that different brand of heat cured acrylic had been used with a different type of nano fillers. On the other hand, an insignificant reduction was observed in transverse strength after immersion the acrylic specimens in 7% of MgO NPs for 30 minutes. Furthermore, there was a highly significant increase in the surface roughness of heat cured PMMA resin upon the incorporation of 1.5% MgO nano fillers. This may be attributed to the difference in roughness between MgO nano fillers and PMMA matrix and also probably related to the difference in micro structural characteristics of PMMA resin and the shape of nanoparticles [29]. The increased surface roughness may provide a strong indicator of good dispersion and extrusion behavior of MgO nano fillers within PMMA matrix. It may also be the result of high residual monomer content in polymer/nanocomposite specimens. The previous explanation is based on some authors' suggestion about the presence of a potential relationship between the amount of residual monomer and the degree of surface roughness in polymerized PMMA dentures [30]. These findings agreed with Aljafery and Hussain (2015) [31]. However, we got a disagreement with those of Jasim (2013) [32]. This may be due to the difference in shape and surface nature of incorporated nano fillers. It was found that the immersion of heat cured acrylic in 7% MgO NPs solution for 30 minutes insignificantly increased the surface roughness of denture base material.

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